In the last few decades, the field of healthcare has been progressing rapidly, offering huge scope and prospect to overcome the restriction of several unresolved challenges. Expertise from diverse fields like material science, bioinformatics, biomedical, electronics and even computer science are incorporating their proficiency to understand the complex biological phenomena, developments of high throughput biosensors and novel therapeutic approaches. Development of the biologically important materials by systematic arrangement of the materials in nanoscale level is one of the emerging areas of research in this regard. This offers a viable option of developing novel nanostructured materials with improved properties owing to its high surface to volume ratio, suitable for drug delivery, bio-imaging, sensing and theranostics. One of the main intentions of nanotechnology involves the development of novel therapeutic materials suitable for biomedical and pharmaceutical applications. Physical and chemical methods used for the synthesis of metal nanoparticles are common and old practices. But these methods associated with high cost chemicals that are toxic and hazardous to both human health and environment (Babu et al. 2011). Therefore, biological methods of NP synthesis were studied widely that are capable of replacing synthetic reducing and stabilizing agents (Babuet al. 2011). Biological methods of synthesis of NPs involve different micro and macroscopic organisms such as bacteria, fungi,
algae and plants. It was reported that NPs synthesized from plants are more stable and the rate of synthesis is faster than that of microorganisms (Iravani 2011). Moreover, as the plants are existing abundantly over the earth, the use of plants materials for synthesis of NPs offer a great deals of scope of large scale production of NPs. The use of plant extract has opened a new era in fast and nontoxic methods for the synthesis of metal NPs (Iravani 2011).

5.1 Plant mediated synthesis of AuNPs

“Let food be your medicine and medicine be your food” is an incentive Hippocrates launched more than 2400 years ago (Hakim 1988), gaining more and more followers nowadays, as people become more aware about the benefits of a healthy living. Medicinal plants are the plants with medicinal properties, i.e. they can be directly or indirectly used for medical purposes. The background principle of this approach is that these plants contain certain biologically active substances that influence the metabolic processes of humans (Muruganatham et al. 2016). Due to the various benefits, medicinal plants have been widely used by the researches for various pharmaceutical and biomedical fields. In nanotechnology also plant mediated synthesis of various metal nanoparticles are widely explored. NPs synthesized from the extracts of different plant parts such as leaves, stems, root, bark, flower, fruits and seeds have been reported to use for the synthesis of metal NPs (Iravani 2011). The various natural compounds and secondary metabolites present in the extracts were suggested to be responsible for bio reduction and stabilization of NPs.
The plant extracts used for this study were bract of *M. balbisiana*, leaves of *C. dactylon*, leaves of *E. foetidum*, leaves of *E. fluctuans*, flower of *C. maxima* and *C. fistula*. These plants have already been studied widely for their photochemical properties.

Somana *et al.* (2008) studied the anthocyanin composition of bracts of *M. balbisiana* and reported two non methylated anthocyanins- delphinidin-3-rutinoside and cyaniding-3-rutinoside (Somana *et al.* 2008). Also, Gogoi *et al.* (2014), studied the antioxidant activity of *M. balbisiana* aqueous and alcoholic extracts of inflorescence. They found the total phenolic content was 5.9± 0.06 mg GAE/g d.w. and total flavonoids content 73.8 mg QE/g d.w. of the alcoholic extracts of inflorescence (Gogoi *et al.* 2014). *M. acuminata*, another member of Musaceae family has been reported to have the antioxidant activity (Sumathy *et al.* 2011). *M. balbisiana* methanolic extract was also reported to have antibacterial activity (Chye *et al.* 2015).

Also the general phytochemical analysis of ethanolic *M. balbisiana* bract extract showed the presence of flavonoids, phenols and anthocyanins, in the present study. These phytochemicals were supposed to be responsible for the synthesis and stabilization of AuNPs (Sumathy *et al.* 2011). Natural flavons have been shown to serve as a reducing agent in AuNPs synthesis (Wang *et al.* 2007). Also the flavonoids showed to prevent agglomeration and stabilization of AuNPs in aqueous solution (Nune *et al.* 2009).

*Cynodon dactylon* was also studied for its phytochemicals composition. Phytochemical analysis of the alcoholic extract of *Cynodon*
showed the presence of glycosides, flavonoids and alkaloids (Garg et al. 2008), phenols, quinines and tannin (Kaleeswaran et al. 2010). The whole plant is reported for its use in the treatment of diuretic, dropsy, syphilis, wound infection and piles (Parekh et al. 2007). Work carried out by Sahu et al. (2013), showed the synthesis of silver NPs from leaf extract of C. dactylon (Sahu et al. 2013). Also the phytochemicals such as flavonoids, polyphenols and tannin present in the extract was suggested to be responsible for the successful reduction of AgNO$_3$ to Ag NPs (Lee et al. 2014). These synthesized AgNPs has showed the antibacterial activity against E. coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella typhimurium. In the present study the ethanolic extract of the C. dactylon was evaluated for the synthesis of AuNPs and results are known to be very promising. General phytochemical analysis also showed the presence of flavonoids and phenols in the extract. Therefore, it can be suggested that the phytochemicals present in the extracts are responsible for the successful synthesis of AuNPs.

Aswathy et al. (2014), studied the composition of E. foetidum and reported E (2) Dodecenal, 2, 3, 6 Trimethylbenzaldehyde as one of the major compounds present in the methanolic extract (Aswathy et al. 2014). The compound E-2-dodecenal was reported as the main components of E. foetidum oil. E. foetidum has been reported to mainly consist of aldehydes, ranging from 45.8% to 86.7%, (Lo et al. 1991, Leclercq et al. 1992, Paul et al. 2011)). The phytochemical studies on the genus indicated the presence of essential oils, flavonoids and coumarins and romarinic acid derivatives (Hohmann et al. 1997, Pala-Paul et al. 2006, Erdelmerier et al. 1985, Le Claire et al. 2005). Chandira
et al. (2012) reported the presence of carbohydrate, glycosides, saponin, polysterols, flavonoids, proteins, phenolic compounds and tannins in the aqueous extract of the plant (Chandira et al. 2012). General phytochemical evaluations of E. foetidum in this present study showed the presence of flavonoids, phenols, tannins and carbohydrates. In the present study, these various phytochemicals are supposed to be present in the leaves extract and responsible for the synthesis and stabilization of AuNPs.

Another plant considered for this study was E. fluctuans. In general, people consume this herb as vegetables in India as well as many Asian countries. Phytochemical study on the plant showed the presence of flavonoids, triterpenes, carbohydrate, reducing sugar, phenols, tannin, saponin and proteins (Kuri et al. 2014). Amin et al. (2012) studied the anti-microbial activity of this plant against against both gram positive (+) and gram negative (-) bacteria as well as three pathogenic fungi and reported satisfactory results (Amin et al. 2012). The antimicrobial activity was suggested due to The presence of phytochemicals (Mandalari et al. 2007, Maiyo et al. 2010, Viji et al. 2010) like alkaloids, steroids, flavonoids, terpenoids, glycosides etc, in the leaves of E. fluctuans. Research carried out by Sannighahi et al. (2011) on the flavonoids content of the plant showed the presence of two major compounds in methanolic extract as baicalein-7-O glycoside and baicalein-7-Odiglucoside. Also the crude methanolic extract showed flavonoids rich fraction and total flavonoids was reported to be 92.32+ 12.35 µg/mg. (Sannighahi et al. 2011).

General phytochemical studies of the plant in the present study also showed the presence of flavonoids, phenol, and tannin. Therefore, it can be
anticipated that these various phytoconstitutents were responsible for the successful reduction and synthesis of AuNPs.

*Curcubita maxima* Duch. (Pumpkin) also could be included among the other medicinal plants due to its dietary benefits (Muruganatham *et al.* 2016, Saha *et al.* 2011b). Pumpkin is also known to possess pharmacological activities like antitumor, anticancer, antidiabetic etc. (Hartwell 1967, Saha *et al.* 2011a, Saha *et al.* 2011c, Saha *et al.* 2011b) Nutritional and health benefit values of pumpkin draw considerable attention to food scientists in recent years (Fokou *et al.* 2004). Pumpkin belongs to the angiosperm family Cucurbitaceae, under the genus *Curcubita* with different varieties (Alfawaz 2004)

Among the known pharmacological activities of pumpkin, a few are, antitumor (Hartwell, 1967) (Saha *et al.* 2011), antiobesity (Das *et al.* 2010), antidiabetic (Saha *et al.* 2011), hepatoprotective (Saha *et al.* 2011), diuretic (Jose *et al.* 2008), antioxidant (Attarde *et al.* 2010), antigenotoxic (Villasefior and Lemon, 1996), vermifuge and taenicide (Al-Rawi and Chakravarty 1964, Burkill *et al.* 1966), remedy for carbuncles (Al-Rawi and Chakravarty 1964), uses in cataplasms (Al-Rawi and Chakravarty 1964, Pitier 1926), tonic (Al-Rawi and Chakravarty 1964), warts cure (Hartwell 1967, Edward *et al.* 2013). Among the various phytochemicals both α and β carotenoids were reported to be present in pumpkin flower (Seroczyńska *et al.* 2006). Flower of *C. maxima* was also considered for its ability to reduced $\text{Au}^{3+}$ ions to AuNPs, in the present
study. General phytochemical analysis showed the presence of flavonoids, anthocyanin and phenols in the ethanolic flower extract of *C. maxima*.

Studies carried out by Bhalodia *et al.* (2011), showed that the hydroalcoholic extract of *Cassia fistula* flowers possessed antioxidant activity (by inhibiting DPPH and hydroxyl radical). Also the preliminary phytochemical investigation indicates the presence of phenols and flavonoids in the plant. In addition, Bhalodia *et al.* (2011) also reported that the hydro alcoholic extract of *C. fistula* found to contain a noticeable amount of total phenols and tannins which play a major role in controlling antioxidants. The results of this study show that the hydroalcoholic extract of *C. fistula* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry (Bhalodia *et al.* 2011). General phytochemical evaluation ethanolic flower extract in the present study also showed the presence of tannin, flavonoids and phenols.

Another member of the genus *Cassia* (*C. auriculata*) which has potent antidiabatic activity was also studied for its gold nanoparticle synthesis. Using aqueous leaf extract of the plant Kumar *et al.* (2011) has reported the synthesis of AuNPs with average size of 15-25 nm (Kumar *et al.* 2011).
5.2 Characterization of AuNPs

5.2.1 UV Visible spectroscopic analysis of synthesized AuNPs

The as-synthesized AuNPs developed using different plant extract-were characterized by using UV-Vis spectroscopy which revealed its characteristics surface plasmon peak. In case of *M. balbisiana* the optimum extract concentration was found to be 7% (w/v) with a narrow absorption peak at 530 nm (Fig: 4.1.2.1). Furthermore increase in the EBE concentration did not always show the increase in intensity. This may be due to the saturation of the Au$^{3+}$ ions present in the reaction solution indicating the complete reduction of 1 mM Au$^{3+}$ ions by plant extract. Das *et al.* (2011) studied the synthesis of AuNPs from different concentrations of *Nyctanthes arbortristis* flower extract. The 7% (w/v) extract concentration was found to be optimum for the AuNP synthesis and beyond 7% (w/v) extract concentration the synthesis occur with negligible increase in the SPR intensities. It implied that beyond 7% (w/v) of plant extract the reaction solution becomes saturated indicating the complete reduction of the 1mM gold ions. This finding of Das *et al.* (2011) was found to be in conformity with the present study.

Similarly in case of *E. foetidum* also different ELE was tested for the synthesis, and here also results found are similar with *M. balbisiana*. Here 7% (w/v) of ELE was found to be optimum as there was not significant increase in the SPR intensities with the increase in the ELE concentration with a sharp SPR
intensity at 531 nm (Fig: 4.1.2.3). Study carried by Daisy Phillip on honey mediated synthesis of gold nanoparticles showed that at lower concentration of honey, the SPR peak was broad with an absorption tail towards the longer wavelength attributing the excitation of the in-plane SPR which indicates significant anisotropic in the shape of AuNPs. As the quantity of honey was increased, SPR goes to the longer wavelength side with sharper absorption peak at 541 nm (Phillip 2009a).

In case of *C. dactylon* (Fig: 4.1.2.2) SPR intensities increased from 1% to 4%. In the 4% of extract concentration there was observed a blue shift. Beyond this the SPR intensities increased with a little red shift. Similarly a blue shift observed in case of *E. fluctuans* at 4% (Fig: 4.1.2.4), *C. maxima* (Fig: 4.1.2.5) at 6% and *C. fistula* at 8% (Fig: 4.1.2.6) (Babu et al. 2013). The SPR peaks of the AuNPs from *C. dactylon* found at 530 nm, *E. fluctuans* found at 539 nm, *C. maxima* found at 538 nm and *C. fistula* found at 532 nm.

At lower concentrations of the extracts (EBE, ELE, EFE) it was observed that synthesis occur at some with a broad SPR intensity (Fig: 4.1.2.1, 4.1.2.3, 4.1.2.5 and 4.1.2.6) which indicated the formation of large anisotropic particles. This is because at lower extract quantities growth is favoured but due to the absence of sufficient biomolecules responsible for capping and efficient stabilization results in large particles (Phillip 2011, Parashar et al. 2009, Babu et al. 2009, Phillip et al. 2009b, Phillip et al. 2010).

While the amount (w/v) of plant extract increased from 1% to 9%, the intensity of the corresponding SPR peak was also increased with blue shifting of SPR peak. This implies that the amount of NPs was increased with slight
alteration of the size of the NPs. As reports suggest that change in size and shape result the shifting of the SPR signal (Das et al. 2011, Noruzi et al. 2011, Ghoreishi et al. 2011). Hyperchromic shift (blue shift) indicates the formation of the smaller NPs. This is possibly due to the higher amount of stabilizing agents, i.e., plant extracts which help in the formation of smaller NPs. This is very significant as it offers the possibility of the large scale production of NPs by using plant extract as stabilizing as well as reducing agent. Moreover, this simple protocol of synthesis of NPs can be opted in order to vary the size of the NPs by changing the amount of plant extract without altering the reaction environment.

Daizy Philip et al. (2010) reported the synthesis of AuNPs from Mangifera indica leaf. They also reported that when quantity of extract increased, the band width of SPR decreased with a shift of the bands towards lowers wavelength (blue shift). This indicated the synthesis of large anisotropic particles. They also reported the similar SPR intensity modulation in case of AuNPs synthesized from edible mushroom, Volvariella volvacea, Murraya koenigii leaf (Phillip et al. 2010). Their results are found similar with the present findings of C. dactylon, E. fluctuans, C. maxima and C. fistula.

5.2.2 Transmission Electron Microscopy (TEM) analysis

AuNPs synthesized from the optimised plant extracts (EBE or ELE or EFE) was taken for the TEM analysis. TEM analysis revealed the abundance of
mostly spherical particles in the samples of AuNPs (Fig: 4.1.3.1, 4.1.3.2, 4.1.3.3, 4.1.3.4, 4.1.3.5, 4.1.3.6).

Selected-area electron diffraction pattern (SADE) confirms the crystalline nature of the AuNPs (Fig: 4.1.3.1 B, 4.1.3.2 B, 4.1.3.3 B, 4.1.3.4 B, 4.1.3.5 B, 4.1.3.6 B). The Debye-Scherrer’s rings corresponding to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) gold crystalline planes were observed of all the cases, indicated the present of FCC packing of Au NPs. Also, the particle size distributions of the particles were calculated, average particle size was found to be around 8 ± 2 nm for AuNPs synthesized from M. balbisiana. Similarly, in case of C. dactylon, E. foetidum, E. fluctuans, C. maxima and C. fistula it was found to be around 37 nm, 27 nm, 12 nm, 19.5 nm and 10 nm.

The high resolution TEM image (HRTEM) also displayed the clear lattice fringes on the particle surface (Fig: 4.1.3.1 C, 4.1.3.2 C, 4.1.3.3 C, 4.1.3.4 C, 4.1.3.4 C, 4.1.3.5 C, 4.1.3.6 C).

Similar results were also reported by Das et al. (2011), in case of N. arbortristis the Debye-Scherrer’s rings corresponding to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1), also HRTEM image display clear lattice fringe on the particle surface. Babu et al. (2011), in case of coconut water mediated AuNPs synthesis also showed the Debye-Scherrer’s rings corresponding to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1). The interplanner distance of Au (1 1 1) plane was in agreement with the (1 1 1) d-spacing of bulk Au (0.2355) (Kannan et al. 2008, Shankar et al. 2004a, Shankar et al. 2004b, Narayanan et al. 2008).
In the present study also HRTEM of the synthesized AuNPs from all the six plant extracts showed the clear lattice structure with d-spacing from 0.20 to 0.22. The SADE patterns with bright ring also corresponds to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes (Shankar et al. 2004a, Narayanan et al. 2008).

### 5.2.3 FTIR analysis

FTIR spectrum of lyophilized EBE of *M. balbisiana* showed peaks at 1036 cm\(^{-1}\) (aliphatic amines), 1306 cm\(^{-1}\) (aromatic amines), 1647 cm\(^{-1}\) (amide), 2934 cm\(^{-1}\) (alkane) and 3579 cm\(^{-1}\) (hydroxyl). After synthesis, these values shifted to 1047 cm\(^{-1}\) (amine), 1399 cm\(^{-1}\) (aromatic amines), 1639 cm\(^{-1}\) (C=C of benzene), 2939 cm\(^{-1}\) (alkane) and 3414 cm\(^{-1}\) (hydroxyl), which confirms interaction of EBE during AuNP synthesis (Fig: 4.4.1)

Similarly, lyophilized ELE of *C. dactylon* showed peaks at 1061 cm\(^{-1}\) (C-N of amines), 1159 cm\(^{-1}\) (C=O of alcohols, carboxylic acid, esters or ether or C-N of amines), 1458 cm\(^{-1}\) (N=O of Nitro), 1747 cm\(^{-1}\) (C=O of aldehydes), 2866 cm\(^{-1}\) (C-H of aldehydes), 2926 cm\(^{-1}\) (C-H of alkanes) and 3431 cm\(^{-1}\) (-OH of alcohols or phenols). After synthesis these values changed to 1069 cm\(^{-1}\), 1169 cm\(^{-1}\), 1461 cm\(^{-1}\), 1738 cm\(^{-1}\), 2928 cm\(^{-1}\) and 3402 cm\(^{-1}\) which confirms interaction of ELE with HAuCl\(_4\) solution during AuNP synthesis.

Lyophilized ELE of *E. foetidum* showed peaks at 1029 cm\(^{-1}\) (C-N of amines), 1379 cm\(^{-1}\) (C-N of amines), 1648 cm\(^{-1}\) (N-H of primary or secondary amines and amides), 2921 cm\(^{-1}\) (C-H stretch of alkane) and 3426 cm\(^{-1}\) (-OH
alcohols or phenols). After synthesis these values changed to 1061 cm\(^{-1}\), 1387 cm\(^{-1}\), 1640 cm\(^{-1}\), 2937 cm\(^{-1}\) and 3417 cm\(^{-1}\) which confirms interaction of ELE with HAuCl\(_4\) solution during AuNP synthesis.

Dried lyophilized ELE of *E. fluctuans* showed peaks at 1020 cm\(^{-1}\) (C-N of amines), 1387 cm\(^{-1}\) (C-N of amines), 1632 cm\(^{-1}\) (N-H of primary or secondary amines or amide), 2912 cm\(^{-1}\) (C-H of alkane) and 3434 cm\(^{-1}\) (-OH of alcohols or phenols). These peak values shifted to 1053 cm\(^{-1}\), 1404 cm\(^{-1}\), 1640 cm\(^{-1}\), 2937 cm\(^{-1}\) and 3402 cm\(^{-1}\) after synthesis.

Dried lyophilized EFE of *C. maxima* showed peaks at 1020 cm\(^{-1}\) (C-N of amines), 1461 cm\(^{-1}\) (N=O of nitro), 1648 cm\(^{-1}\) (C=C of alkanes), 1730 cm\(^{-1}\) (C=O of esters or carboxylic acid), 2831 cm\(^{-1}\) (C-H of aldehydes), 2921 cm\(^{-1}\) (C-H of alkane) and 3419 cm\(^{-1}\) (-OH of alcohols or phenols). These values changed to 1061 cm\(^{-1}\), 1406 cm\(^{-1}\), 1624 cm\(^{-1}\), 2928 cm\(^{-1}\), and 3404 cm\(^{-1}\) after synthesis.

Similarly, lyophilized EFE of *C. fistula* showed peaks at 1012 cm\(^{-1}\) (C-N of amine), 1232 cm\(^{-1}\) (C-O of alcohols or carboxylic acid), 1461 cm\(^{-1}\) (N=O of nitro), 1510 cm\(^{-1}\) (C=C of aromatic amine), 1632 cm\(^{-1}\) (C=C of alkanes), 2912 cm\(^{-1}\) (C-H of alkane), 3410 cm\(^{-1}\) (-OH of alcohols or phenols). These values changed to 1069 cm\(^{-1}\), 1240 cm\(^{-1}\), 1455 cm\(^{-1}\), 1518, cm\(^{-1}\) 1616 cm\(^{-1}\), 1743 cm\(^{-1}\), 2928 cm\(^{-1}\) and 3420 cm\(^{-1}\) after synthesis.
Gan et al. (2012) has reported the synthesis of AuNPs from palm oil mill effluent (POME), representing a low cost and viable approach of AuNPs synthesis. They had studied the FTIR spectra of both POME and AuNPs. 2351 cm\(^{-1}\) peak of N-H or C=O stretching vibrations band of POME changed to 2376 cm\(^{-1}\). This phenomenon revealed that biomolecules in POME such as proteins or flavonoids that contain abundant of C=O or N–H groups might play an important role in the bio reduction and stabilization of AuNPs during the synthetic process. Also, FTIR of AuNPs shows bands at 1056 cm\(^{-1}\) and 1645 cm\(^{-1}\) which are C-N stretching of aliphatic amines or phenols and amide I bands respectively. Another band at 2376 cm\(^{-1}\) and 2974 cm\(^{-1}\) are characteristics of N-H or C=O and C-H stretching vibrations. In addition, the band at 3453 cm\(^{-1}\) indicates the presence of polyphenolic -OH group. Hence, Gan et al. (2012) deduced that AuNPs might be stabilized and capped by functional groups present in the POME, such as proteins and polyphenols through the interactions of free amine groups in the proteins as well as the carbonyl groups in the polyphenols (Gan et al. 2012).

Song et al. (2009) studied the synthesis of AuNPs from Magnolia kobus and Diopyros kaki leaf extract. They used FTIR analysis for the characterization of the extract and the synthesized nanoparticles. The FTIR spectra of the M. kobus leaf extract before and after synthesis of AuNPs did not show any significant changes. The FTIR spectrum of the leaf extract showed bands at 3332 cm\(^{-1}\) and 1637 cm\(^{-1}\). The intense broad absorbance at 3332 cm\(^{-1}\) is the characteristic of the hydroxyl functional group in alcohols and phenolic compounds (Song et al. 2009).
The band at 1637 cm\(^{-1}\) can be assigned to the amide I band of the proteins released by the Magnolia leaves or to C=C groups/aromatic rings. The FTIR spectrum of the gold nanoparticles showed bands at 1024 cm\(^{-1}\), 1227 cm\(^{-1}\), 1629 cm\(^{-1}\), 1736 cm\(^{-1}\), and 2916 cm\(^{-1}\) along with other small bands. The band at 1024 cm\(^{-1}\) corresponds to the C–N stretching vibration of aliphatic amines or to alcohols/phenols. The weaker bands at 1227 cm\(^{-1}\) and 1629 cm\(^{-1}\) correspond to the amide III and amide I bands of proteins, respectively. The bands at 1736 cm\(^{-1}\) and 2916 cm\(^{-1}\) can be assigned to the carbonyl groups and secondary amines, respectively. This indicates that gold nanoparticles synthesized using the M. kobus extract are surrounded by some proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, and carboxylic acids (Song et al. 2009).

Raghunandan et al. (2009) studied the AuNPs synthesis from guava (Psidium guajava) leaf extracts. They reported that the probable pathway of biosynthesis using Fourier Transformed Infrared spectroscopy. FTIR peaks at 1704 cm\(^{-1}\), 1607 cm\(^{-1}\), 1385 cm\(^{-1}\), 1197 cm\(^{-1}\), and 1082 cm\(^{-1}\) represent the different functional groups of the adsorbed biomolecules on the surface of the AuNP. This also suggests the influence of other organic moieties in the synthesis and stabilization of AuNP such as flavonoids (Sugany et al. 2007) and terpenoids (Begum et al. 2004), which are abundant in guava leaves, showed characteristic absorption peaks that appear to be responsible for accelerated reduction and capping process of AuNPs synthesis. The IR absorptions peaks are noted as follows: 1197 cm\(^{-1}\) and 1,082 cm\(^{-1}\) (–O–), 1385 cm\(^{-1}\) (geminal methyl group, 1607 cm\(^{-1}\) (C=C, aromatic) and 1704 cm\(^{-1}\) (α, β unsaturated
ketoone and esters). The peaks observed suggested the presence of flavonones adsorbed on the surface of the AuNP (Raghunandan et al. 2009).

Daizy Phillip (2010) has reported the synthesis of gold and silver NPs from Hibiscus rosa sinensis. The IR bands of the hibiscus leaf at 1317 cm\(^{-1}\) and 1733 cm\(^{-1}\), which were reported to be characteristics of C-O and C=O stretching modes of carboxylic acid group, possibly of mallic acid present in it (Narayanan et al. 2008, Phillip et al. 2009a, Shankar et al. 2003a, Shankar et al. 2003b, Ankamwar et al. 2005b, Lin et al. 2005). Peaks at 1619 cm\(^{-1}\) and 1546 cm\(^{-1}\) were suggested to be of amide I and amide II bands. These bands were due to N-H deformation vibrations in the amide linkages of the proteins (Phillip 2009b, Basavaraja et al. 2008, Ahmed et al. 2003c, Shankar et al. 2004a, Solomun et al. 2004) present in it. The peaks at 1399 cm\(^{-1}\) was the C-N stretching mode of aromatic amine groups (Narayanan et al. 2008) and at 1022 cm\(^{-1}\) was due to the C-O-C and C-OH vibrations of protein in the leaf extract (Phillip 2009a, Phillip 2009b). After synthesis peaks were observed at 1638 cm\(^{-1}\) and 1721 cm\(^{-1}\). The amide I band has shifted to higher frequency 1638 cm\(^{-1}\) compared to that of plain leaf 1619 cm\(^{-1}\) and amide II band become more prominent. The peak 1721 cm\(^{-1}\) was due to stretch in C=O bands of amide I and amide II indicated the possibility that AuNPs are bound to proteins through free amine groups. It was also reported as a well known fact that the proteins can bind to AuNPs through the free amine groups or carboxyl ion of amino acid residue in it (Ankamwar et al. 2005b, Ahmed et al. 2003a, Ahmed et al. 2003c, Huang et al. 2007).
Huang et al. (2007) has studied the pharmaceutical components of the *Cinnamomum camphora* leaf showed that alkaloids, flavones, steroids, terpenoids, coumarins, linalools, lactones, hydroxybenzenes, anthracenes, polysaccharides, amino acids, and proteins exist in such a leaf (Su et al. 2006 and Gao 2003). They also reported the synthesis of AuNPs and AgNPs from *C. camphora* leaf. Before bioreduction, the FTIR absorption spectra of the dried biomass of *C. camphora* leaf showed absorbance bands centred at 1109 cm\(^{-1}\), 1244 cm\(^{-1}\), 1317 cm\(^{-1}\), 1384 cm\(^{-1}\), 1446 cm\(^{-1}\), 1517 cm\(^{-1}\), 1631 cm\(^{-1}\) and 1726 cm\(^{-1}\) observed in the region 1000–1800 cm\(^{-1}\). Among them, the absorbance bands at 1109 cm\(^{-1}\), 1631 cm\(^{-1}\) and 1726 cm\(^{-1}\) were suggested to be associated with the stretch vibration of \(\text{–C–O, C=C,RHC=O}\), respectively (Zhu 2000). To a large extent, the band at 1109 cm\(^{-1}\) might be due to the \(\text{–C–O}\) groups of the polyols such as flavones, polysaccharides, and terpenoids in the biomass. But the disappearance of the band at 1109 cm\(^{-1}\) after bioreduction shows that the polyols were thought to be mainly responsible for the reduction of chloroaurate ions or silver ions. It is speculated that the alcohol groups are oxidized to carbonyl groups, thus leading to the band 1726 cm\(^{-1}\). All in all, it can be suggested that the water-soluble fractions in the biomass played an important role in the bioreduction and shape evolution of the nanoparticles.

The FTIR spectra of the obtained nanoparticles manifest several absorption peaks located in the region 1000–2000 cm\(^{-1}\), which were 1042 cm\(^{-1}\), 1077 cm\(^{-1}\), 1384 cm\(^{-1}\), 1606 cm\(^{-1}\), 1622 cm\(^{-1}\), 1715 cm\(^{-1}\), and 1762 cm\(^{-1}\).
two absorption peaks located at around 1042 cm\(^{-1}\) and 1077 cm\(^{-1}\) can be assigned as the absorption peaks of \(-\text{C}–\text{O}–\text{C}–\) or \(-\text{C}–\text{O}–\). Also, the broad absorption spectra at about 1606 cm\(^{-1}\) and 1622 cm\(^{-1}\) might result from the stretching vibration of \(-\text{C}=\text{C}–\). In addition, they also reported some weak absorption spectra in the region 1700 cm\(^{-1}\) – 1800 cm\(^{-1}\), which might be due to the stretching vibration of \(-\text{C}=\text{O}\). Studies showed that, the bonds or functional groups, such as \(-\text{C}–\text{O}–\text{C}–\), \(-\text{C}–\text{O}–\), \(-\text{C}=\text{C}–\) and \(-\text{C}=\text{O}\), derive from the heterocyclic compounds that were present as water-soluble components in the biomass (Pavia \textit{et al.} 2001) (Zhu 2000). Therefore, it was thought that the heterocyclic compounds, such as alkaloids, anthracenes and flavones, were responsible for the capping of the nanoparticles (Zhu 2000).

From the above discussions, it was found that the present study resembles with the similar works carried out by different researchers in this field. The changes in the FTIR peaks in the present study were a confirmation of the interaction of the different plant components with HAuCl\(_4\) solution.

\textbf{5.2.4 XRD analysis}

XRD pattern of the AuNPs synthesized from \textit{M. balbisiana} showed four prominent Bragg reflections, which were indexed on the basis of fcc structure of gold. The intensities as confirmed from JCPdf 00-001-1172, of the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) diffraction peaks were corresponded to 38.2\(^{\circ}\), 45.2\(^{\circ}\), 65.8\(^{\circ}\) and 78.0\(^{\circ}\) respectively and confirmed the crystalline nature of the
synthesized AuNPs (Fig: 4.5.1). Similarly, in case of AuNPs synthesized from 
*C. dactylon*, it was also observed that diffraction peaks occur at 38.4°, 44.2°, 
62.9° which corresponds to the (1 1 1), (2 0 0), and (2 2 0) planes of fcc gold 
(Fig: 4.5.2). AuNPs synthesized from *E. foetidum*, showed diffraction peaks at 
38.4° and 44.7° which correspond to (1 1 1) and (2 0 0) planes (Fig: 4.5.3). 
AuNPs from *E. fluctuans* showed peaks at 38.08° and 44.5° which corresponds 
to (1 1 1) and (2 0 0) planes (Fig: 4.5.4). AuNPs from *C. maxima* and *C. fistula* 
showed prominent peaks at 38.34° and 38.10° which corresponds to (1 1 1) 
plane of gold fcc structure. Also small peaks were found at 44.2° for *C. maxima* 
and 44.5° and 64.4° for *C. fistula*, which corresponds to (2 0 0) and (2 2 0) 
planes of fcc gold (Fig: 4.5.5, 4.5.6).

The present findings are found related with the reports of many 
researchers. Das *et al.* (2011), reported the XRD diffraction peaks of 
synthesized AuNPs from *N. arbortristis* at 38.1°, 44.4°, 64.8° and 78° 
corresponding to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of fcc lattice 
structure of gold. Similar results were also reported by Babu *et al.* (2011). They 
found XRD peaks at 38.1°, 44.5°, and 64.8° for synthesized AuNPs (Das *et al.* 

Noruzi *et al.* (2011) also reported the XRD peaks at 38.4° (1 1 1), 
44.6° (2 0 0), 64.8° (2 2 0), 77.6° (3 1 1) and 82.09° (2 2 2). Dubey *et al.*(2010) 
reported the synthesis of AuNPs from leaf extracts of *Rosa regusa* and
observed XRD peaks at $38.1^0 (1\ 1\ 1)$, $64.5^0 (2\ 2\ 0)$ and $77.4^0 (3\ 1\ 1)$ (Noruzi et al. 2011 and Dubey et al. 2010b)

Gan et al. (2012), reported similar observations of XRD peaks at $38.1^0 (1\ 1\ 1)$, $44.1^0 (2\ 0\ 0)$, $64.7^0 (2\ 2\ 0)$ and $77.8^0 (3\ 1\ 1)$ of synthesized AuNPs from palm oil mill effluent (Gan et al. 2012).

Dubey et al. (2010), reported the AuNPs synthesized from Sorbus aucuparia and observed XRD peaks at $38.21^0 (1\ 1\ 1)$, $44.39^0 (2\ 0\ 0)$, $64.62^0 (2\ 2\ 0)$ and $77.59^0 (3\ 1\ 1)$ (Dubey et al. 2010a). From these reported literature the present findings are found to be similar. Therefore, XRD results of the present study support the synthesis of AuNPs from the plants extracts taken for the study.

5.3 Characterization of the synthesized drug loaded composite NP (AuNPCHCS)

Khandelia et al. (2013) had studied the protein agglomerates as the versatile nanocarriers for drug delivery. They had synthesized the citrate stabilized AuNPs using well known citrate reduction of HAuCl$_4$ with small modification of previous report (Jimenez et al. 2011). Nanocarriers generated based on the agglomeration of AuNPs with lysozyme in aqueous medium were found to be efficient in order to encapsulate both hydrophobic (doxorubicin) and hydrophilic (pyrene). Then these nanocarriers were coated with albumin layer for their better stability and studied for their uptake by cancer cells. The
UV-Vis spectra of the AuNPs and the agglomerate showed changes in the SPR intensities indicating the non-covalent interactions of the agglomerates with drug molecules.

Similar techniques were adopted here to achieve the encapsulation of the drug molecules within agglomeration of AuNPs. The agglomeration of AuNPs was induced by a biodegradable, cationic polymer chitosan (CH), while cisplatin (CS) - a very common anticancer drug was taken as a model drug molecules to find out the efficiency of the composite NPs. CH is used in many studies for reduction as well as capping of AuNPs synthesis. Chitosan (CH) is a naturally occurring polysaccharide composed of (1, 4)-linked amino-2-deoxy-β-D-glucose and 2-acetamido-2-deoxy glucose units. The polymer was reported to have significant amount of primary amines and hydroxyl groups which provides its strong affinity towards metal ions. The affinity of the CH towards the metal ion can easily be incorporated by simple chelation or by ion exchange making it an excellent support for synthesis of metal nanoparticles or for stabilization of synthesized metal nanoparticles (Hardy et al. 2004, Mallick et al. 2012).

Vigderman et al. (2013) also reported the therapeutic platforms where gold nanoparticles were covalently conjugated with drug molecules. They reviewed platinum based drug (cisplatin) nanoparticle conjugates used as drug delivery vehicle and a prodrug for intracellular release of platinum ions (Vigderman et al. 2013, Dhar et al. 2008a, Dhar et al. 2008b, Feazell et al. 2007). Min et al.(2010) synthesized Pt (IV) prodrug to gold nanorods, which were first covalently functionalised with polyethylene glycol using in situ
dithiocarbamate formation on one end and second amino group for coupling with carboxyl-containing platinum (IV) compound. MTT based cytotoxicity of these tested on 3 cancer cell lines, which showed a much higher toxicity compared to free cisplatin, with IC_{50} values ranging from 9 to 65 times lower. This indicates that the NPs mediated drug delivery vehicle can enhance the cellular uptake of the drug via endocytic pathway (Min et al. 2010, Tong et al. 2007).

Sahoo et al. (2014), reported the development of a composite consisting of highly fluorescent AuNP nanostructure and the biopolymer chitosan, that could easily be converted into NPs and would form a stable polyplex with suicide gene for induction of apoptosis in cervical cancer cells (Sahoo et al. 2014). Mallic et al. (2012), has studied the synthesis of a new composite of copper nanoparticles (CuNPs) and chitosan and studied their antibacterial activity against gram negative bacteria *E. coli* and gram positive bacteria *Bacillus cereus*. The CuNP with CH composite was attached to the bacterial cell wall which causes irreversible damage of the membrane, eventually leading to the cell death (Mallick et al. 2012).

In the present study, the AuNPs and drug loaded composite NPs (AuNPCHCS) were studied using UV-Vis spectroscopy. A shift in the absorption spectrum of AuNPs observed after addition of CH which was indicating the formation of agglomeration of AuNPs and CH. Initially, AuNPs has absorption maxima at 530 nm which changes to 532nm on addition of CH, which further changes to 534 nm on addition of CS (Fig: 4.2.1). Though the changes in the absorbance wavelength were indicative of interactions of the
AuNPs with the added molecules, additionally the small changes in the SPR spectra also suggest that there were no major alterations of the agglomerated nanostructures present in the medium. It was also reported that AuNPs are known to absorb a specific light band with absorption peak ranging from 520 nm to 540 nm due to surface plasmon resonance or SPR (Aryal et al. 2009).

The position of the maximum peak and the width of the absorption band depends on the morphology of the particles (size, shape and uniformity), coagulation among the particles and the dielectric environment (Daniel et al. 2004, Dutta et al. 2004, Liz-Marzan 2004, Link and Sayed, 2003).

Aryal et al. (2009) also reported the stability of the thiol stabilized AuNPs and doxorubicin conjugates up to 3 months at room temperature. In the present study also both the synthesized AuNPs and AuNPCHCS shows stability up to one month at room temperature and there was no significant difference in the spectra of the two (Fig: 4.2.1.2).

Jang et al. (2013) studied the nuclear delivery of doxorubicin from dextran-coated AuNPs. They had aminated the citrate synthesized AuNPs for better stability for various biological applications. Here also it can be observed that red shift in the UV-Vis spectra of AuNPs with the addition of molecules. These findings of Jang et al. (2013) were also in conformation with the present findings (Jang et al. 2013).

DLS based study was also conducted to analyse the particle diameter. Khandelia et al. (2013) had reported the average hydrodynamic diameter of the synthesized AuNPs as 19 nm, which changes to 116 nm on addition of
lysozyme and further to 195 nm on addition of doxorubicin. Jang et al. (2013) had also analysed the size of the AuNPs and reported that the DLS spectra shifted to higher wavelength indicating increase in the overall size. These findings are found to be similar with the present study. Here the DLS based diameter of the AuNPs found to be 63.59 nm and composite to be 97 nm which is suitable for cellular uptakes. Additionally, the hydrodynamic diameter was found to be favourable for passive targeting by exploring the EPR effect of tumour microenvironment.

Tomulesa et al. (2011) had studied the effect of AuNP conjugated with cisplatin, doxorubicin and capecitabine on hepatocellular carcinoma derived cancer cells. The cell viability was found to be lower in presence of the anticancer drugs delivered by AuNPs compared to the drug alone. They reported the synthesis of the AuNPs using L-aspartate as reducing and capping agent. Further, the aspartate nanostructures were functionalized with the desired drugs. The TEM analysis of the structures involving the AuNPs has been rearranged in the presence of the drug and maintains a nanometric size (Tomulesa et al. 2011, Khandelia et al. 2013). This study was also found to be similar with the present study. In the present findings AuNPCHCS structures observed was bigger than the only AuNPs. AuNPs sizes from TEM image were calculated to be around 8nm, while AuNPCHCS was found to be around 82 nm (diameter of the particles were calculated using image j software) (Fig: 4.2.3).

Tomulesa et al. (2011) also studied the FTIR analysis of the nanoconjugates. After binding the drug to the AuNPs, the band, observed at the range of 3200-3700cm⁻¹, which were due to the presence of surface bound –N
and –OH groups. The peak shifting from 2980 cm\(^{-1}\) to 2978 cm\(^{-1}\) represented the stretching vibrations of C-H bonds from the rings. In the present study, the FTIR peaks of AuNPs (\textit{M. balbisiana}) found at 1047 cm\(^{-1}\) (amine), 1399 cm\(^{-1}\) (aromatic amines), 1639 cm\(^{-1}\) (C=C of benzene or N-H stretching of primary amine and amide bands), 2939 cm\(^{-1}\) (alkane) and 3414 cm\(^{-1}\) (hydroxyl). Addition of chitosan (CH) to these AuNPs shows peaks at 1058 cm\(^{-1}\), 1643 cm\(^{-1}\), 2940 cm\(^{-1}\) and 3420 cm\(^{-1}\). As mentioned earlier, chitosan (CH) is also reach in primary amines and hydroxyl group (Hardy \textit{et al.} 2004). Again addition of cisplatin (CS) shows peaks at 1079 cm\(^{-1}\), 1403 cm\(^{-1}\), 1653 cm\(^{-1}\), 2953 cm\(^{-1}\) and 3479 cm\(^{-1}\) (Fig: 4.2.2). This peaks shifting showed the interaction of CH and CS with AuNPs and formation of composites NPs. It was observed that the stretching in the amine and the amide rich regions as well as the hydroxyl regions was involved in the synthesis and agglomeration of the composites NPs. Therefore, it can be suggested that the flavonoids and phenolic groups present in the plant extracts might be responsible for the synthesis as well as agglomeration of both AuNPs and AuNPCHCS.

Surface charge is also one of the important parameter for effective cellular uptakes of NPs. Thus, we have carried out Zeta potential analysis of the AuNPs synthesized from \textit{M. balbisiana} and it was found to be -30 mV, while zeta potential of AuNPCHCS was found to be 23.1 mV. This increased zetapotiveintial is due to addition of cationic chitosan and cispltin which offers the plusibility of electrostatic interection with the negetivey charged plasma membrane. Madhusudan \textit{et al.} (2014), also studied the loadind and releasing of anticancer drug doxorubicin from CM (Carboxymethyl)-Chitosan stabilized
AuNP. They reported the zeta potential value of CM-Chitosan reduce AuNPs was -21.6 mV where, doxorubin loaded AuNPs was -10.6 mV. Doxorubicin is a positively charged drug and Zeta value towards positive side indicates the successful interaction between the drug and the AuNPs (Madhusudan et al. 2014). Similarly cisplatin was also a positively charged drug and the changes in the charge of the particles confirm the interaction of the AuNPs with CS in the present study (Fig: 4.2.5).

One of the important parameters for good composite NPs for drug delivery would be the ability to release drug efficiently in a controllable manner at the target site. In the present study in vitro Drug release trend of the composite NPs was studied at physiological and acidic conditions such as PBS (pH 7.4) and acetate buffer (pH 5.6) at 37°C. Drug released percentage at 24 h was found to be 12.05% in PBS and 21.3% in acetate buffer (pH 5.6) respectively (Fig: 4.3). It is well documented that cancer cells have an extracellular acidic environment (Bhujwalla et al. 2002) and most of anticancer drug such as doxorubicin, cisplatin etc at free state are not effectively taken up at acidic conditions (Sheetal et al. 2011). Since many cancer cells have low intracellular pH (Tannock et al. 1989), drug should be able to work at that low pH within the cells. In the present study, it was observed that the drug release from the composite NPs in the acidic environment was comparatively more than in physiological condition. At physiological conditions drug loaded composite NPs showed stability as it is necessary for the transportation of the drug with minimal loss (Madhusudan et al. 2014, Jayasuriya et al. 2013, Khandelia et al. 2013).
5.4 Cytotoxicity tests of synthesized Gold nanoparticles (AuNPs)

Synthesized gold nanoparticles from all six of the plant extracts were tested for their cytotoxicity in a cancerous cell line, HeLa and primary cell line, CEF up to a concentration of 100 µM using MTT assay [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium)]. Further the significance of the experiment was studied by statistical analysis. The values of all experiments were expressed as mean ± standard deviation (SD).

In case of HeLa cells, AuNPs synthesized from all the six plant extracts showed high cell viabilities. Cells retain their normal morphology suggesting that the AuNPs did not cause any cytotoxic effect on the cells and therefore can be used for further drug delivery assays. In case of AuNPs synthesized from *M. balbisiana* it was found that at 100 µM concentration the cell viability was recorded as 92.9% (Fig: 4.4.1). Similarly AuNPs synthesized from *C. dactylon, E. foetidum, E. fluctuans, C. maxima* and *C. fistula* the cell viability up to 100 µM were found to be 92.8%, 89.4%, 92.4%, 90.3% and 91.8% respectively (Fig: 4.4.2, 4.4.3, 4.4.4, 4.4.5, 4.4.6).

In case of CEF cells AuNPs synthesized from all the six plant extracts were also tested. Here also normal morphology of the cells observed suggested that the AuNPs did not cause any cytotoxic effect on the cells and therefore can be used for further drug delivery assays. In case of AuNPs synthesized from *M. balbisiana* it was found that at 100 µM concentration the cell viability was recorded as 91.2% (Fig: 4.4.7). Similarly AuNPs from *C dactylon, E foetidum, E fluctuans, C maxima* and *C fistula* the cell viability percents up to 100 µM...
were found to be 88.2\%, 89.8\%, 92.8\%, 90\% and 87.4\% respectively. (Fig: 4.4.8, 4.4.9, 4.4.10, 4.4.11, 4.4.12)

A similar study was reported by Babu et al. (2011), where they quantify the cytotoxic effect of biosynthesized AuNPs from coconut water. Up to a concentration of 100 µM, cell viabilities observed were 87\% in HeLa and 85\% in MCF-7 cancer cell lines. Both cell lines retained their original cellular morphology, suggesting that the AuNPs did not induce any cytotoxic effect (Babu et al. 2011). They also studied the cytotoxicity of AuNPs synthesized from Bacopa monnieri leaf extract, by MTT assay and found AuNPs were biocompatible when tested on HeLa and MCF-7 cell lines (Babu et al. 2013).

5.5 Cytotoxicity tests of synthesized drug loaded composite NPs (AuNPCHCS)

Cytotoxicity tests of the synthesized composite NPs (AuNPCHCS) were studied by both qualitatively and quantitatively.

5.5.1 Qualitative test

Cytotoxicity tests of the synthesized composite NPs (AuNPCHCS) were qualitatively studied by simply under inverted light microscopy and Mac-Grunwald Giemsa staining of monolayer cells.
5.5.1.1 Observation under inverted light microscopy

In Fig 4.5.1.1.1 (A) shows the monolayer of HeLa cells without treatment and Fig 4.5.1.1.1 (B) shows the same cells treated with 10 µg/ml concentration of composite NPs. It was observed that the monolayer was not intact and also the cell morphology was distorted after treatment for 24 h. Fig 4.5.1.1.2 shows the CEF cells (A) without treatment and (B) with treatment. Here in Fig 4.5.1.1.2 (A) healthy monolayer of CEF observed with long spindle shaped cells and Fig 4.5.1.1.2 (B) shows the distorted monolayer with rounded dead cells.

5.5.1.2 Mac-Grunwald Giemsa staining of cells

Chen et al. (2009) had studied the assessment of in vivo toxicity of AuNPs on BALB/C mice. One of the results of the studies of toxicity was H and E staining (Haematoxylin-eosine staining) of the major organs. The staining showed significant differences in the treated and untreated organs. Similarly in the present study also Mac-Grunwald Giemsa staining of cells showed the differences in the composite NPs treated and untreated cells (HeLa and CEF). In the staining photographs as mentioned in results (Chapter 4), Fig 4.5.1.2.1 (A) showed healthy HeLa cell monolayer with prominent nuclei. Cytoplasm is stained blue and nuclei are dark blue. Fig 4.5.1.2.1 (B) showed treated (10µg/ml) HeLa cells, with no differentiation between cytoplasm and nuclei. Also, cell morphology was distorted which confirms the cytotoxic activity of the composite NPs. Similar results were also observed in case of
CEF cells. Fig 4.5.1.2.2 (A) showed healthy CEF monolayer with proper spindle shaped cells and 4.5.1.2.2 (B) showed treated cells (10 µg/mL) with distorted and discontinuous cells.

5.5.2 Quantitative test – MTT Assay (3-(4,5—dimethylthiazol-2-yl)-2,5-diphenyltetrazolium)

Cell proliferation and death are essential for any cytotoxicity study and multiple procedures are available for such study. Pharmaceutical and biomedical research largely focuses on the effects of drugs, cytotoxic agents and biologically active compounds which effect cytogenesis. MTT assay is one of the commonly used cytotoxicity assay to evaluate the cell cytotoxicity. Khullar et al. (2012), studied the synthesis and cytotoxicity of bioconjugated AuNPs with BSA (bovine serum albumin) towards cancer cell line (Glioma cell line- aN-nitrosomethyl urea induced cell line of rat brain). They had also studied the cytotoxic effect by using MTT assay (Khullar et al. 2012).

Kumar et al. (2008), studied the biosynthesis of AuNPs using a plant pathogenic fungus (Helminthosporium solani) and their conjugation with doxorubicin on HEK293 cells (Human embryonic kidney 293 cells) using MTT assay (Kumar et al. 2008b). Similar studies by Aryal et al. (2009) had also shown the use of MTT based cell viability assay for the cytotoxicity study of doxorubicin conjugated AuNPs (Aryal et al. 2009).

AuNPs synthesized from all the six plant sources shows cell viability more than 80% in both the cell lines i.e. HeLa and CEF. Among all these,
AuNPs from *M. balbisiana* showed 92.9% in HeLa and 91.2% in CEF and was taken for the composite NPs synthesis.

In **HeLa** cell line, the composite of AuNP with chitosan (CH) showed cell viability similar to control (without treatment). Therefore, it can be suggested that the AuNPCH was non toxic in nature (Fig: 4.5.2.1). With the addition of the anticancer drug cisplatin (CS) to the AuNPCH, there was observed a gradual decrease in the cell viabilities. At the 10 µg/mL concentration of the composite NPs -AuNPCHCS cell viability was found to be 52%.

Fig 4.5.2.3 shows the cell viability of **HeLa** at varying concentrations, of composite NPs and free drug. It was observed that when the cells were incubated with 0.67 µg/mL of composite, 84% cells were viable. At the same concentration, in case of free drug, 88.4% cells were viable.

At the concentration of 10 µg/mL, cell viability reduced to 52% in case of composite NPs and 55.7% in case of free drug (CS). These findings suggested that when non toxic composite NPs (AuNPCH) were loaded with drug (CS), were able to kill the cancer cells efficiently compared to free drug.

In case of **CEF** also, the composite AuNPCH showed cell viability similar to control (without treatment) indicating the AuNPCH was non toxic in nature (Fig: 4.5.2.2). With addition of the anticancer drug cisplatin (CS) to the AuNPCH there observed a gradual decrease in the cell viabilities. At the 10 µg/mL concentration of the composite NPs -AuNPCHCS cell viability was found to be 49%.
Similarly, Fig 4.5.2.4 also suggested that AuNPCHCS composite NPs were able to kill the primary cells, CEF. At the concentration of 0.67 µg/mL, cell viability was 78.2% in case of composite NPs and 83.5% in case of free drug (CS). Here also, at the concentration of 10 µg/mL, cell viability observed was 49.9% in case of composite NPs and 52.1% in free drug (CS). The results were similar to that of cancer cell line.

Further, the significance of the experiment was studied by statistical analysis. One way ANOVA was done but no significant difference in the treatment was found. But it was important to be noted here that composite NPs exhibit similar cytotoxic effect as free drug which is essential, as in many cases it get compromised after loading with nanoparticles or nanoparticles mediate carriers. Thus, the composite NPs synthesized in the present study as the same or with more suitable modifications may offer the possibility of its use in passive targeting by exploring the EPR effect, or in photothermal therapy as were nontoxic to cells.

5.5.3 SEM analysis

SEM image of HeLa cells as well as CEF cells treated with the composite NPs shows the cell rupture and the formation of apoptotic bodies on the cell surface while control cells shows smooth surface of the healthy cell (Fig: 4.6.1, Fig: 4.6.2). Similar results were reported by Khandelia et al. (2013), had studied the effect of anticancer drug doxorubicin loaded AuNPs functionalized with lysozyme nanocarriers on HeLa cells. SEM image showed
cytoplasm blebbing on the cell surface. These are the characteristics of the apoptotic cells (Khandelia et al. 2013). Also Moirangthem et al. (2012) studied the effect of *Cephalotaxus griffithii* extract on HeLa cell line, and cytotoxicity showed similar cell blebbing of the cells (Moirangthem et al. 2012). Sahoo et al. (2014) studied the fluorescent Au nanocluster with chitosan (CH) for the delivery of suicidal gene in the HeLa cells. SEM results obtained showed apoptotic body on treated cells (Sahoo et al. 2014).

### 5.5.4 DNA fragmentation study

DNA fragmentation study was done to observed the fragmentation or laddering pattern in the treated cells. This a signature of late apoptosis event which is characterised by nuclear fragmentation.

In case of Hela untreated cells (control), there was observed a prominent band without any smearing. But in case of the treated cells (IC₅₀, 10µg/mL) in the lane, there observed a shearing (Fig: 4.7.1). This could be due to the nuclear damage because of the treatment. In case of the CEF, similar result was observed. Treated CEF cells (IC₅₀, 10 µg/mL) lane showed fragmentation (Fig: 4.7.2). Moirangthem et al. (2012) studied the effect of *Cephalotaxus griffithii* extract on HeLa cell line, and cytotoxicity also studied by DNA fragmentation assay (Moirangthem et al. 2012). The control cells showed no fragmentation while treated cell DNA was found to be fragmented. Similar results were also observed in the present study. Treated HeLa cells DNA showed shearing and CEF cells showed fragmentation. DNA
fragmentation was a hallmark of late apoptosis. Therefore, from the present study it can be suggested that the composite NPs developed are able to kill the cancer as well as primary cells in apoptotic way. Though the activity of the composite NPs was not much different from the single drug, it is important to be noted that composite exhibited similar cytotoxic effect as free drug, which is essential as many cases it got compromised after loading with composite NPs. Thus, this composite NPs mediated delivery offers the likelihood of passive targeting by exploring the EPR effect.

Moreover, the composite NPs are comprised with AuNPs, which has unique advantage such as biocompatibility. It can be used as contrast agent in TEM, which will help in probing the endocytosis process, or it can also be suitably used in photo thermal processes.

5.6 Future perspective of the synthesized AuNPCHCS

The use of nanoparticles and nanoparticle mediated therapeutics are rapidly progressing and are being implemented to solve several limitations of conventional drug delivery systems, such as poor bio distribution and targeting of the drug, lack of water solubility etc. To improve the bioavailability of the cancer drugs to the targeted area NPs are designed and modified based on their size and surface characteristics. NPs offers the possibility to encapsulate poorly soluble drugs (Kipp 2004, Zhang et al. 2008), protect therapeutic molecules (Whitehead et al. 2009), modify their blood circulation and tissue distribution (Alexis et al. 2008, Bertrand et al. 2012). These properties of NPs are known to
be attractive in oncology to encapsulate the anticancer drugs exhibiting wide range of toxicity and physiochemical properties.

The size and the surface characteristics of NPs reported to play an important effect on drug delivery to the targeted tumor tissue (Cho et al. 2008). Nanoparticles for an effective drug delivery must have the ability to remain in the bloodstream for a considerable time without being eliminated. Wisse et al. (1996) reported that surface non modified NPs that are conventionally used usually caught in the circulation by the reticuloendothelial system, such as the liver and the spleen, depending on their size and surface characteristics. Therefore, it was suggested that by adjusting their size and surface characteristics NPs can be controlled for drug delivery.

One of the advantages of NPs is that their size is tuneable. The size of nanoparticles should be large enough to prevent their rapid leakage into blood capillaries but small enough to escape capture by macrophages that are present in the reticuloendothelial system, such as the liver and spleen, for a good drug delivery system. The size of the sinusoid in the spleen and fenestra of the Kuffer cells in the liver varies from 150 to 200 nm (Wisse et al. 1996) and the size of gap junction between endothelial cells of the leaky tumor vasculature may vary from 100 to 600 nm (Yuan et al. 1995). Consequently, the size of nanoparticles should be up to 100 nm to reach tumor tissues by passing through these two particular vascular structures.
In addition to their size, the surface characteristics of nanoparticles are also an important factor determining their life span and fate during circulation relating to their capture by macrophages. Nanoparticles should ideally have a hydrophilic surface to escape macrophage capture (Moghimi et al. 2003). This can be achieved in two ways: coating the surface of nanoparticles with a hydrophilic polymer, such as PEG, protects them from opsonisation by repelling plasma proteins; alternatively, nanoparticles can be formed from block copolymers with hydrophilic and hydrophobic domains (Harris et al. 2001, Adams et al. 2003).

**Passive targeting** by NPs is the accumulation of NPs functionalised with drug in the tumor site for long time that facilitates the slow and targeted release of the drug, which constitute an important mechanism of drug delivery in tumor cells. The accumulation of NPs functionalised with NPs or macromolecules with molecular weight above 50KDa on the tumor site is facilitates due to some unique pathophyologic features of the tumor vessels (Maeda 2001a, Maeda 2001b). The fast growing cancer cells develop new vessels (neovascularisation) or reroute the existing vessels near tumor mass to supply them with oxygen and nutrients (Carmeliet 2000). These results in the development of highly disorganized and dilated vessels of the tumor with numerous pores showing enlarge gap junctions between endothelial cells and compromised lymphatic drainage (Carmeliet 2000). These type of features are known as “enhanced permeability effect” or in short EPR effect, which constitute an important mechanism in drug delivery in the tumour site.
Matsumura and Maeda (1986) were first to report on the EPR. They observed the tumor accumulation of therapeutic macromolecules, poly (Styrene-co-Maleic Acid)-NeoCarzinoStatin(SMANCS), a 16kDa polymer conjugate that non covalently binds albumin in the circulation to reach a molecular weight of around80 kDa(Maeda 2001b, Kobayashi and Maeda 1988). The distribution of SMANCS to the tumor vicinity was observed in early preclinical development and led to further investigate the phenomenon (Matsumura and Maeda 1986). Using labelled albumin and other proteins in addition to the polymer conjugate, they showed that proteins larger than 30 kDa (i.e. SMANCS), murine and bovine albumins (67 and 69 kDa, respectively) and IgGs (160 kDa) could preferentially distribute to the tumor interstitium and remain there for prolonged periods of time (Matsumura and Maeda 1986). EPR effect had been reported by Fang et al. (2011) as one of the good standards for the macromolecular anticancer drug design (Fang et al. 2011).

Another popular area in nanomedicine is the implementation of gold nanoparticles in photothermal therapy (PTT), a minimal invasive therapy in which photon energy is converted into heat to kill the cancer cells. AuNPs absorb light strongly and converted photon energy into heat quickly and efficiently, which makes them superior contrast agent for PTT (Huang et al. 2011a, Huang et al. 2011b). They are very photostable and biocompatible and these features make them a new generation PTT agents for photothermal therapy to induce cellular damage via thermal effect such as hypothermia, coagulation and evaporation (Svaasand et al. 1990, Goldman 1967, Goldman et
The use of nanosecond pulsed laser PTT is highly selective and localised damage controllable from a few nanometers to tens of micrometers depending on the laser pulse duration and particle size (Pustovalvo et al. 2008).

Lin and co workers in 2003 were first to study the selective and highly localised photothermolysis of targeted lymphocyte cells by the use of pulsed laser and gold nanospheres (Pitsillides et al. 2003). AuNPs conjugated with antibodies were incubated with the lymphocytes cells and exposed to nanosecond laser pulses (Q-switched Nd: YAG laser, 565 nm wavelength, 20 ns duration) showed cell death with 100 laser pulses at an energy of 0.5 J/cm². Adjacent cells just a few micrometers away without nanoparticles remained viable. Their numerical calculations showed that the peak temperature lasting for nanoseconds under a single pulse exceeds 2000 K at a fluence of 0.5 J/cm² with a heat fluid layer of 15 nm. The cell death was attributed mainly to the cavitation damage induced by the generated micro-scale bubbles, around the nanoparticles (Huang et al. 2011, Pitsillides et al. 2003). Similar studies were also reported on the photothermal destruction of K562 cancer cells (Zarov et al. 2003).

For any ideal anticancer drug it is important to have first, the ability to reach the desired tumor tissue through the penetration of barriers of body with minimal loss of their volume and activity. Secondly, after reaching the tumor tissue they should have the ability to selectively kill the tumor cells without affecting the normal cells in a controlled release mechanism of the drug.
Increasing research shows NPs seem to have the potential to satisfy both of these requirements for effective drug carrier systems.

In the present study, the size of the AuNPCHCS conjugate was found in the range of 80-100 nm by DLS and TEM analysis. Moreover, the composites also, showed good cytotoxic effects in both HeLa (IC$_{50}$ value 10 µg/mL) and CEF (IC$_{50}$ value 10 µg/mL) cells, which was similar to that of free drug. Therefore, it was observed from the obtained results that the composite synthesized could be used for development of a novel drug delivery system, which will help to probe the endocytosis process, or it can also be suitably used in photo thermal processes.