Plants are used to cure human diseases due to the presence of phytoconstituents, which are medicinally significant. The phytoconstituents present in the plants are non-nutritive and responsible for the physiological actions in humans. The development of drugs from this phytoconstituents is becoming important in recent years for several ailments especially for Diabetes, use of these synthetic hypoglycemic drugs leads to many side effects. Hence, the present study was carried out to evaluate the antioxidant, antibacterial and hypoglycemic activity of *C. anisata* extracts (leaf, root, SNP leaf and SNP root) under *in vitro* and *in vivo*.

Simple maceration method was performed in this study as it is preferred to be simple, reliable, gives good and selective extraction and requires limited amount of solvent (Walton and Brown, 1999). The presence of the phytoconstituents tannins, alkaloid, steroids, saponins, phenolics, flavonoids and cardiac glycosides in the ethanolic leaf extract of *C. anisata* was corroborated with the study of Agyare et al., (2014). Another study also reported that the ethanolic root extract contains tannins, flavonoids, alkaloids and saponins (Kenechukwu *et al.*, 2012; Afolayan *et al.*, 2015). The presence of phytoconstituents in plants may help for its survival and to carry out its basic functions such as symbiosis, competition, metal transport and differentiation (Demain and Fang, 2000). Its is scientifically proven that these bioactive compounds have anticancer, antioxidant, anti diabetic, anti viral, anti cancer, anti malarial, antidiuretic, anti-inflammatory anti bacterial, anti fungal activities, and act as analgesic, known to reduce coronary heart disease, cholesterol etc., but these effects are still unknown, currently their effects are
investigated and researched (Mir et al., 2013; Saxena et al., 2013). Hence the phytochemical screening is used to predict the different types of potential phytoconstituents from plants that may lead to drug discovery and development.

Ethanolic leaf extract of *C. anisata* showed similar antioxidant activity that was reported by Agyare *et al.*, (2015) with IC$_{50}$ value of 32.9 µg/ml that may be due to the phenolic compounds, flavonoids and flavanols present in this plant. The antioxidant activity in leaf essential oil of *C. anisata* showed concentration dependent inhibition of DPPH radical with EC$_{50}$ value of 2.66 mg/l (Goudoum *et al.*, 2013). When the free radical concentration exceeds the level of antioxidant mechanism it leads to various diseases including DM. Free radicals or ROS generated may damage the cell through covalent binding and lipid peroxidation with leads to tissue injury. Before attacking the target biological cells, antioxidants will stabilize the free radicals. If it cannot, the ROS will induce oxidative stress that may lead to disintegration of cell membrane and cause damage to lipid, protein, DNA, which ultimately results in the development of chronic and degenerative diseases. Thus the presence of antioxidant in the plants may help to protect against the diseases they induce in biological system. The medicinal plants that have high level of antioxidants not only fight against various diseases but also act as an effective therapeutic approach for hepatic damage (Khan *et al.*, 2013; Khan *et al.*, 2012).

A simple, reliable DPPH method was carried out to evaluate the antioxidant activity of different extracts of *C. anisata*. Alloxan which is known to induce experimental diabetes in animals, cause necrosis of β-cells and induces free radical generations H$_2$O$_2$, .OH and O$_2$. that cause cell damage followed by cell death (Abdul-Hamid and Moustafa, 2013).
The antibacterial activity for crude extracts and SNPs synthesized using extracts of *C. anisata* was similar to previous report of Chelvi *et al.*, (2015) who revealed that SNPs from aqueous leaf extract of *M. emarginata* was active against *P. seruginosa, E. coli, B. cereus, S. aureus* and *E. coli* where highest MIC was found for *B. cereus* with 100 µg/ml and Afolayan *et al.*, (2015) carried out a similar study on antibacterial activity of leaf acetone extract of *C. anisata* that was active against both gram positive and gram negative bacteria with MIC 0.1 mg/ml – 0.5 mg/ml. The dichloromethane extract of the bark was active against *S. aureus, E. coli, Streptococcus pyogenes* with MIC 5 mg/ml. The aqueous extract did not showed any activity against the tested organisms (Lawal *et al.*, 2015; Mukandiwa *et al.*, 2012).

The secondary metabolites in plants are most widely used in agriculture, medicine, pharmaceutical industry and also act as natural antibiotic that inhibit the growth of microorganisms (Clavo *et al.*, 2002; Mapleston *et al.*, 1992; Stone and Williams, 1992). The phytoconstituents such as coumarins, quinines, terpenoids, essential oil, tannins, flavonoids, alkaloids, phenanthrene, phenolic acids, polyphenols and other aromatic compounds act as a defense mechanisms against several microorganisms, insects and herbivores (Doughari, 2006; Cowan, 1999; Bouarab *et al.*, 2009). Foot infection is common in diabetic patients. The organisms used in this was selected based on the previous reports of Bansal *et al.*, (2016) who isolated the presence of *P. aeruginosa, E. coli, S. aureus, K. pneumonia* and *Proteus vulgaris* out of 157 organisms from the diabetic patients with foot lesions and gram negative organisms was found to be predominantly present in the lesions. Several studies showed the presence of *Clostridium perfringers, Staphylococcus epidermis* and *Bacillus subtilis* along with other gram positive
and gram negative organisms in diabetic wound (Alam et al., 2014; Aragon-Sanchez, 2010; Bowler et al., 2001; Mukandiwa et al., 2012).

The difference in the activities of antibacterial test, for gram positive and gram negative bacteria is due to the morphological difference in the cell wall and membrane organization. Due to this difference, the gram positive bacteria are more susceptible to phytoconstituents compared to gram negative bacteria, in which a combined phytoconstituents is needed to provide a synergistic antibacterial activity against gram negative organisms (Bouarab et al., 2009). Depending on their solubility or polarity of the solvents, different solvents have the ability to extract different phytoconstituents. In this study, the ethanolic extracts might have the higher solubility rate for phytoconstituents that leads to highest antibacterial activity.

In case of SNPs, silver itself having antimicrobial activity against wide range of bacteria, fungi and viruses. When silver is used in nitrate form, it induces antimicrobial effect. But when SNPs are used, high surface area will be available for the microbes that are exposed to (Prabhu and Pouluse, 2012). Gram positive bacteria are less susceptible to Ag$^+$ than gram negative bacteria that may be due to the thick peptidoglycan layer in gram positive bacteria that bears negative charge than gram negative bacteria (Ikram et al., 2016). In this study, SNP showed efficient antimicrobial activity compared to the other extracts that may be due to the extremely large surface area that provides better contact with test organisms.

The result suggests that alpha amylase was inhibited by most polar components of C. anisata which is similar with another study that reported aqueous and methanolic extracts of C. anisata inhibited human urinary
amyase with IC$_{50}$ values of 1947±50 and 2436±62 mg/ml respectively (Mogale et al., 2012). The results obtained for SNP extracts of leaf and root in this study was in line with earlier report, methanolic extract of SNPs obtained from *Costus pictus* that showed IC$_{50}$ value 534.39 µg/ml, where acarbose showed 513.97 µg/ml (Aruna et al., 2014).

The hallmark of DM is hyperglycemia, produced by glucose after few minutes of ingestion. The carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for breaking α-1, 4 – bonds in disaccharides and polysaccharides, thereby liberating glucose and maltose that leads to hyperglycemia. The liberated glucose diffuse to intestinal epithelial cells, where they are taken by the passive diffusion, facilitated diffusion, through transporters (GLUT) and by co-transport with other ions (Na$^{2+}$) (Urooj et al., 2014). The plant extract should have the ability to control glucose liberation from starch and its absorption. This may act as a therapeutic modality in the management of diabetes by reducing post prandial glucose level (Mahomoodally et al., 2014; Muthuvel et al., 2013).

From the results it was clear that, when alpha amylase, glucose and *C. anisata* extracts where taken together the extract may inhibit the carbohydrate metabolizing enzyme activity, in which flavonoid was reported to have the ability to inhibit the alpha amylase (Tadera et al., 2006).

The results obtained for glucose uptake by yeast cells was in accordance with Aruna et al., (2014) who reported that the rate of glucose uptake into yeast cell was linear in 5 mM and 10 mM glucose concentration for *Costus pictus* methanolic extract with IC$_{50}$ 94.09 µg/ml and 84.26µg/ml respectively, where SNPs of this extract showed 78.39 and 69.87 µg/ml at
5mM and 10mM respectively. And also similar to the methanolic extracts of *Artocarpus heterophyllus* that exhibited high glucose uptake at 10 mM glucose concentration with 78.42% at 2000 µg/ml (Nair *et al.*, 2013). The glucose can be readily absorbed across the plasma membrane of most cells. Several papers have reported that in yeast cells, the glucose transport is mediated by stereospecific membrane carriers and also by facilitated diffusion. These facilitated carriers, transport solutes down the concentration gradient (Urooj *et al.*, 2014).

The results obtained was in accordance with another study who reported that methanolic extract of *Psoralea corylifolia* showed gradual increase in glucose concentration with time in external solution and decrease in concentration within the dialysis membrane. The concentration of glucose varied from 80-860 µg/ml (Sagadevan *et al.*, 2014; Devi and Das, 2015). This may also be due to the inhibition of enzyme α-amylase, which inhibits the release of glucose from starch, due to the high concentration of fibre, viscous polysaccharides, encapsulation of enzyme and starch that reduce its accessibility (Devi and Das, 2015; Ahmed *et al.*, 2011). In another study it is reported that polyphenolic compounds have the ability to bind with enzyme protein and hence it may inhibit digestive enzymes (Mahomoodally *et al.*, 2014). The result was corroborated with Devi and Das (2015) stated that glucose adsorption capacity was directly proportional to the molar concentration of glucose.

The difference in *C. anisata* leaf and root glucose adsorption rate may be due their affinity to bind substrate and total dietary fibre content. The dietary fibres have the ability to bind glucose and showed adsorption capacity for glucose, only when the concentration of glucose increases (Ou *et al.*, 2014).
The glucose adsorption that occurred at lower concentration of glucose (5 mMol/l), may reduce the glucose transport across the intestinal lumen, thereby reducing the postprandial hyperglycemia (Devi and Das, 2015).

The phytoconstituents in plants play an important role as reducing and capping agents that stabilize the SNPs to prevent agglomeration and increase in particle size (Phatak and Hendre, 2015). The mechanism of synthesis of *C. anisata* SNPs under sunlight irradiation may be due to solar photos that hit the NPs present in the solution during exposure under sunlight. The dissolved oxygen molecules in the solution will react with the excited electrons at the particle surface and get converted into oxygen anion radicals (Phatak and Hendre, 2015). The change in color of the reaction was due to splitting of AgNO₃ to Ag⁺ and NO⁻₃, as time progress. The reduction in Ag⁺ ions into Ag may be due to the metabolites in the leaf and root extracts acted as electron donor. Due to the excitation of surface plasmon vibrations, the formation of SNPs was indicated by brown color of the aqueous solution.

Similar to this study, Arunachalam and Rastogi (2011) reported the synthesis of SNPs using aqueous garlic extract by exposing under bright sunlight for 15 mins and it was found to be stable for a very long period and retained their bactericidal potential. The biosynthesis of SNPs from aqueous leaf extract of *Synedrella nodiflora* under sunlight irradiation was also reported by Jash *et al.*, 2014, that produced a peak centered near 428.7 nm.

UV-Visible spectroscopy is an indirect method for determining the reduction of silver ions to SNPs. The optical phenomenon is due to the plasmon resonance in silver and gold metals. The SPR band is due to the free electrons in the conduction band due to small particle size (Daisy and Saipriya, 2012). Due to the combination of proteins, aminoacids, enzymes,
polysaccharides, alkaloids, tannins, phenolics, saponins and terpenoids may be responsible for the reduction and stabilization of silver ions (Ikram et al., 2016). Proteins that have stronger binding affinity to SNPs will increase the stability of synthesized NPs (Phatak and Hendre, 2015).

The absorbance peaks obtained for C. anisata leaf and root extracts was in line with Das et al., (2014) who investigated the synthesis of SNPs from Musa balbisiana, Azadirachta indica and Ocimum tenuiflorum extracts, which possessed a characteristic absorption peaked in the range of 425-475 nm confirmed the reduction of silver ions due to SPR. Another study carried out by Rudra et al., (2013) on the extracts of Securinega leucopyrus showed SNPs with absorbance peaks within the range of 470-490 nm, which was corroborated with this study.

Earlier report suggested that peaks denoting –C-N stretching vibrations of amine, C-O-C, ether linkage, -C-O-, germinal methyl, -C=C- groups were from aromatic rings and alkyne bonds and responsible for compounds like alkaloid, flavonoid and terpenoids that may be act as capping and stabilizing agent for SNPs (Das et al., 2014 and Shameli et al., 2012).

In this study the stability of SNPs may be due to the interaction of hydrogen bond and electrostatic interaction between the capping molecules bound to the SNPs. The SNPs were not in direct contact that indicates that capping agents has stabilized the NPs. The larger SNPs may be due to the aggregation of the smaller ones.

The same type of results was found in SNPs synthesized from stem bark extract of S. alternifolium that showed particle size ranging from 4-48nm sized spherical shaped particles (Yugandhar et al., 2015). The SNPs synthesized from leaf extract of Bixa orellana showed size ranging from 35-65
nm (Tamilselvi et al., 2013). The SEM results are also consistent with those of Annamalai et al., (2011) who reported that synthesis of SNPs from aqueous leaf extract of *Phyllanthus amarus* showed particle ranging between 32-53 nm.

In this study, the other weak signal corresponding to O₂, Cl, and Ca which could have derived from the plant extract and due to biomolecules bound to the surface of the SNPs. Carbon and copper peaks may be due to the presence in the grid. This was consistent with previous studies (Sengottaiyan et al., 2015 and Das et al., 2014). It was reported that NPs synthesized using plant extracts may have a thin layer of capping organic material surrounding the NPs that was obtained from the plant leaf or root extracts. Here, the several Braggs reflection peak was pointed towards crystal structure of silver. The few unassigned Braggs peak might be due to capping agent stabilizing the NPs with were consistent with the earlier report (Malik et al., 2014).

The similar compound 18, 19-seco-15α-yohimban-19-oic acid, 16, 17, 20, 21-tetra-16α-(hydroxymethyl)-, methyl ester was reported to be active on endocrine and reproductive system, that belongs to the family yohimbine. It was also reported to be effective in relieving male impotency and known to exhibit cardiovascular activity (Ogunlesi et al., 2010). Yohimban is a parent structure of indole alkaloid. Based on the structural skeleton the yohimbe alkaloids can be divided into four categories namely yohimbane, heteroyohimbane, corynan and corynoxane alkaloids. Indole alkaloids found in yohimbe extracts are (α, β, allo, dehydrated), corynanthein, dehydrated corynanthein and corynanthin (Smet, 1997).
Corynan-17ol, 18, 19-didehydro-10-methoxy- was reported to have activities such as lipid metabolism regulator, hormone agonist, antidiabetic symptomatic, in diabetic retinopathy treatment and act as G-protein coupled receptor kinase inhibitor that was analyzed by Gurumurthy et al., (2015) in the methanolic extracts of polyherbal formulations of *Syzygium cumini*, *Picrorhisa kurroa*, *Madhuca indica* and *Commiphoramukul* using GC-MS. The biological activity was predicted with the help of prediction activity spectra for substance technique.

In the animal study, there was an increase in the body weight in animals treated with SNP extracts than in those treated with the crude extracts. The results also corroborated with Kumar et al., (2011) that showed the treatment with *D. indica* methanolic leaf extract in alloxan induced diabetic rats at a dose of 250 mg/kg bw and 500 mg/kg bw significantly increased the body weight of animals. This may be due to the reversal antagonism of SNP extracts in controlling muscle wasting and preventing protein loss. The weight loss may be due to loss of tissue proteins that acts as an important sign in diabetes (Juarez-Rojop et al., 2012). The structural proteins were also play an important role in body weight. The decrease in body weight in diabetic rat may also due to loss or degradation of structural proteins in diabetes (Ananthi et al., 2003).

This result agrees with previous observations of Juarez-Rojop et al., (2012) that have employed this model in different plant extract and also reported loss of body weight. The decreased blood glucose level may be due to insulin secretion from beta cells and increased the transport of blood glucose to peripheral tissues. Thus the results are in accordance with Daisy and Saipriya, (2012), who reported that increase in insulin secretion had decreased
the blood glucose level in alloxan, induced diabetes rats treated with gold nanoparticles obtained from aqueous extracts of *Cassia fistula*. Another study stated that *W. fructicosa* crude extract decreased blood glucose level on 7th, 14th and 21st day of treatment in alloxan induced diabetic rats (Qureshi and Abbas, 2013) and administration of curcumin for 2 months reduced the blood glucose level to 110.46±5.589 mg/dl and post prandial glucose level to 160.982±6.784 mg/dl as compared with diabetic control group (Abdul-Hamid and Moustafa, 2013).

From the results obtained, the SNP extracts was found to be most effective after glibenclamide in decreasing the blood glucose. But the values did not reach the normal value at the end of the experimental period, but prolong intake of extracts may bring down the values to normal. Rajasekaran and Kalaichelvan, (2014) reported the similar lipid lowering effects of *Costus pictus* extracts to be more effective against oxidative stress, blood glucose and serum markers.

Triacylglycerol and cholesterol are the principal lipids carried by lipoproteins. Decreased level of triacylglycerol and cholesterol in liver will leads to decreased synthesis of lipoproteins (Bako et al., 2014). Hypercholesteremia and hypertriglyceridemia are known to occur in diabetic rats. The increased concentration of cholesterol will develop atherosclerosis (Ananthan et al., 2003). The reduction of blood glucose level in alloxan induced diabetic rats was similar to the study carried out by Ezika et al., (2010) in which the methanolic extract of aerial parts of *Phyllanthus niruri* decreased the blood glucose level in alloxan induced diabetic rats. It is therefore necessary to control the blood glucose level to prevent and reverse diabetic complications so that it improves the quality of diabetic patient (Ezike et al., 2010).
The result obtained in this study was corroborated with another study where *D. indica* methanolic extract increased protein level in diabetic rats gradually (Kumar *et al.*, 2011). The protein levels in tissue depend on the balance between their synthesis and catabolism from the body. The estimation of protein acts as a clinical marker in diabetic nephropathy. The decrease in protein synthesis in all the tissue is due to deficiency of insulin in alloxan induced diabetic rats (Ananthi *et al.*, 2003).

Devaki *et al.*, (2014) stated that the decrease in protein level in diabetic rats was due to localized damage in the ER which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis. The increased protein level in treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis. Hence from this, it is clear that the administration of *C. anisata* extracts may enhance the protein synthesis by stabilizing the endoplasmic reticulum. The increase in protein may be due to increase in the insulin mediated amino acids uptake, enhancement of protein synthesis or inhibition of protein degradation (Mohan *et al.*, 2014). ALP acts as a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissues being studied. It assesses the integrity of the plasma membrane. Aspartate and amino transferase are present within the liver, heart, gill, kidney, muscles and other organs. These enzymes monitor the liver cytolysis. The presence of this enzyme in serum reflects information on organ dysfunction. AST and ALT are released from damaged liver cells (Quershi and Abbas, 2013).

The decrease in liver enzyme was noticed after the administration of different extracts of *C. anisata* as compared to diabetic rats. It implies the normal functioning and protective effect of *C. anisata* liver and supports hepato protective nature of *C. anisata*. The liver enzyme activities were
related to the diabetic complications such as retinopathy and neuropathy (Rajaram, 2013). The rise in serum AST and ALP is due to the liver damage. AST and ALT are released when injury involves organelles such as mitochondria. ALP is a hydrolase enzyme located in the cytoplasm and is responsible for removing phosphate from nucleotides and proteins released due to hepatic cellular damage. SNP are believed to play a protective role in decrease of transaminase levels.

The results obtained are in line with those of Rajasekaran et al., 2014 who reported an inhibitory effect of different plant extracts on transaminase and showed decrease in increased level of liver enzymes in a dose dependent manner when treated with *D. indica* methanolic extract respectively. *Woodfordia fruticosa* plant extract leads to the decrease in serum ALT, AST and ALP which was similar to this study (Qureshi and Abbas, 2013).

Glucose-6-phosphatase catalyses the final step in gluconeogenesis and glycogenolysis, that hydrolyse glucose-6-phosphate and maintains the blood glucose homeostasis. In Calvin and gluconeogenesis cycle, fructose-1, 6-bisphosphatase converts fructose-1, 6-bisphosphate to fructose-6-phosphate. These two enzymes are regulatory enzymes in gluconeogenesis. Insulin decreases the activity of enzymes such as glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenol pyruvate carboxy kinase and pyruvate carboxylase (Ragavan and Krishnakumari, 2006).

Increase in glucose-6-phosphatase and fructose-6-phosphatase is due to activation or increased synthesis of the enzymes that leads to increased glucose production in diabetes. Similar reports stated that plant extracts treated animals may modulate and regulate the activities of these two enzymes either through regulating cAMP or inhibiting gluconeogenesis (Ananthi et al., 2003).
Insulin decreases the gluconeogenesis process by decreasing the activity of glucose-6-phosphatase and fructose-1, 6-bisphosphatase. In C. anisata these two enzymes were significantly reduced in liver. This may be due to increased insulin secretion which is responsible for the repression of the gluconeogenic enzymes which is already reported in Ragavan and Krishnakumari, 2006 who evaluated the antidiabetic activity in bark extract of T. arjuna using alloxan induced diabetic rats. The enzyme involved in the control of blood glucose homeostasis is glucokinase. It is expressed in liver and control hepatic glucose disposal and also acts as a sensor for insulin secretion in β-cells. The defective regulation of hepatic glucose metabolism leads to type II diabetes (Agius, 2009 and Hosseini-Zijoud et al., 2013). This enzyme catalyzes the first step in liver for glucose utilization. To stimulate insulin secretion, glucose must be metabolized in β-cell and phosphorylated before being utilized by cells.

The plant extracts are reported to raise the hepatic glucokinase activity to a sufficient level to achieve adequate glycemic control in diabetes, causing excessive elevation of triglycerides in plasma and liver by stimulating glycolysis and lipogenesis (Agius, 2009). Earlier reports suggested that the decrease in glucokinase activity in alloxan –diabetic rats may be due to insulin deficiency. Treatment with plant extracts or glibenclamide may increase the activity of this enzyme in liver that may be due to stimulation of insulin secretion that activated glucokinase, thereby increasing the utilization of glucose and thus results in decreased blood sugar level (Anathi et al., 2003; Ragavan and Krishnakumari, 2006). In liver, the expression of glucokinase is dependent on the presence of insulin. If glucokinase increases in liver, it leads to enhanced glycolysis and hepatic glucose uptake.
Alloxan induces diabetes by liberating oxygen free radicals that cause lipid peroxide mediated pancreatic injury. Increase in the lipid peroxidation levels may be indicative of a decrease in the enzymatic antioxidant defense mechanism (Rajaram, 2013). There are many reports stating that free radicals are generated in diabetic beta cells and many antioxidant enzymes play an important role in protecting cells from oxidative damage.

In this study, it was observed that the SNP root extract could increase the GSH and LPO activity in the liver tissue of diabetic rats. This indicates that the extract may inhibit or reduce the damaged pancreas and stimulate the secretion of pancreatic insulin mean time (Hosseini-Zijoud et al., 2013). When antioxidant mechanism are no longer able to react with the free radical generation, LPO affects the cellular integrity, in which antioxidant could retain the detoxification machinery (Anwer et al., 2012).

Oxidative stress (LPO) occurs in cells and tissues at low level. The generation of increased production of free radicals will act on lipids causing lipid peroxidation. Enzymatic and non-enzymatic antioxidants will reduce the free radical concentration in cells and prevent excessive oxidative stress (Al-Shebly and Mansour, 2012). Oxidative stress in diabetes may be partly responsible for the development of diabetic complications. Several studies stated that both free radical production and antioxidant defense are disturbed in diabetes (Lyons, 1991).

One of the major intracellular antioxidant is glutathione. The redox balance of the cells was maintained by glutathione, which is a non-protein thiol in living organisms (Mohan et al., 2014) and other thiols, thereby preventing the oxidative damage. GSH-Px oxidizes GSH to GSSG and reduces hydrogen peroxide to water. The oxidized GSSG is reduced to GSH
by GSH-red, to maintain the balance between GSH and GSSG (Al-Shebly and Mansour, 2012). The enzymes responsible for destruction of peroxides are glutathione peroxidase and superoxide dismutase, they both play an important role in protecting tissue against oxidative damage. Hence the assessment of oxidative stress markers will give information about oxidant and antioxidant status in diabetes (Mohan et al., 2012).

In liver, the free fatty acids are catabolized the acetyl coA and the excess acetyl coA is converted to cholesterol, triglycerides and ketone bodies resulting in ketosis. The abnormally high concentration of serum lipoprotein in the diabetic control rats may also be due to the increase in mobilization of the free fatty acids from peripheral fat depots by glucagons in the absence of insulin. Excess fatty acids in plasma produced by the alloxan-induced diabetes promote the liver conversion of some fatty acids into triglycerol, phospholipids and cholesterol which may be discharged into the blood as lipoproteins (Bako et al., 2014). Hydroxyl radicals are the major active species that cause lipid peroxidation and significant biological damage (Devaki et al., 2014).

On administration of C. anisata extracts to diabetic rats for 30 days significantly reversed these values to near normal. This may be due to the increase in insulin secretion by C. anisata which decrease the total cholesterol and total triglycerides and increase in HDL level. The result also in line with Ezike et al., 2010 in which the methanolic extract of Phyllanthus niruri in normal and alloxan induced diabetic rats reduced the total cholesterol and triglyceride level in dose dependent manner. The dietary or drug therapy reduction of total cholesterol and triglyceride level seems to be beneficial in preventing diabetic complications and also it improve lipid metabolism.
The coronary artery disease in diabetic patients is due to abnormal lipid level. Defect in insulin secretion and its action leads to enhanced lipid metabolism from the adipose tissue to plasma. Insulin play an important role in regulating lipid and lipoprotein metabolizing enzymes, when insulin is deficient it changes the enzyme activity and also affecting the metabolism. The changes in lipid and lipoprotein in diabetic rats is due to defect in insulin secretion and action (Manoharan and Sophia, 2007).

The result obtained for insulin assay, was in line with Manoharan and Sophia, (2007) in which the ethanolic bark extract of *F. racemosa* in alloxan induced rats showed increase in blood glucose level and significant decrease in plasma insulin level in diabetic animals as compared to control. After treatment with extract the blood glucose and plasma insulin level was normal in diabetic rats. Insulin and glucose level in blood act as primary markers of diabetes. Alloxan causes lesion in islets of langerhans that leads to decrease amount of insulin secretion from beta cell (Khan and Shah, 2014).

The alloxan causes free radical damages to the pancreas (Kumar *et al.*, 2011) that destroy insulin reducing beta cells (Ezike *et al.*, 2010) in a dose dependent manner. The hypoglycemic effect of the extract was reported to protect the pancreas from the harmful effects of chronic hyperglycemia. Through antioxidant and hypoglycemic effects the plant extract was known to produce direct tissue repair effect. Generally the total beta cell mass was balanced between the renewal and loss of the beta cell (Ibrahim *et al.*, 2010).

The results were similar with another study, in which the pancreas of normal control animals showed normal islet where diabetic animals showed destruction of beta cells. The treatment with plant extract increased the number of islets as compared to that of diabetic animals (Latha *et al.*, 2009;
Pawar et al., 2011). The ethanolic extract of *C. occidentalis* in diabetic rats showed partial restoration of normal cell population and size of islet cell (Pawar et al., 2011).

The possible mechanism of *C. anisata* for its hypoglycemic action may be by insulin secretion from beta cells of islets, due to increased transport of glucose to peripheral tissue. The methanolic extract of *Vinca rosea* (500 mg/kg bw) was found to be effective in regeneration of beta cell after 14 days of treatment (Ibrahim et al., 2010). The supplementation of aqueous flower extracts of *Vinca rosea* revealed the restoration of size of islet along with beta cell repair. The recovery of beta cell was found to be dose dependent. In this context, a number of plants have also been reported to have hypoglycemic action and insulin stimulating effect.

The *C. anisata* SNP root extracts reduced the serum triglycerides, which was high in alloxan diabetic control. The extract also found to have beneficial effect on plasma insulin and glucokinase activity. Based on the overwhelming evidences, it was clear that hyperglycemia is coupled with hyperlipidemia which leads to cardiovascular diseases (Ananthan et al., 2003). These findings strongly suggests that phytoconstituent of plant origin have antidiabetic effects. The extracts will be further subjected to bioactivity guided drug discovery to isolate the lead compounds responsible for hypoglycemic action and possible mechanisms of action.
SUMMARY AND CONCLUSION

In the view of the several beneficial effects of *C. anisata* in various pathological conditions and with no reports on the mechanisms of their hypoglycemic effects, the present study was designed to compare and inquire the possible effects of crude extracts and SNP synthesized using the extracts on *in vitro* antioxidant, antibacterial, hypoglycemic activity and *in vivo* hypoglycemic activity against alloxan induced wistar strains of albino rats.

The different extracts obtained from leaves and roots of *C. anisata* using solvents of varying polarities such as hexane, combined ethylacetate/chloroform, acetone, ethanol and aqueous was subjected to phytochemical screening. When compared with all the extracts, the ethanolic leaf and root extract showed the presence of major phytoconstituents such as alkaloids, flavonoids, carbohydrates, coumarins, gums and mucilages, additionally saponins was also found to be present only in ethanolic leaf extract.

The free radical scavenging potential of crude extracts and SNP extracts was evaluated by DPPH. Amongst the individual *C. anisata* extracts evaluated, the SNP root extract of *C. anisata* showed maximum DPPH scavenging activity with 74.07% inhibition. Highest percentage of inhibition indicates the better antioxidant potential. Hence, SNP root extract showed better DPPH scavenging activity (74.07%) as compared to the individual plant extracts, where standard showed 75%. Furthermore, both crude extracts and SNP extracts demonstrated antimicrobial activity against bacterial strains used
as evidenced by disc diffusion and agar well diffusion methods. Comparatively the SNP root extract showed the maximum antibacterial activity against the test organisms used at 500 µg/ml in a dose dependent manner.

The *in vitro* hypoglycemic potential of *C. anisata* was assessed by α-amylase inhibition assay and glucose uptake by yeast cells. Based on the results, the ethanolic leaf and root extracts alone evaluated for glucose diffusion inhibitory assay and glucose adsorption assay. The alpha amylase inhibition rate was found to be maximum for SNP root with 83.60% at 500 µg/ml. In presence of *C. anisata* ethanolic root extract the yeast cells has up taken glucose with 88.50% at 25 mM glucose concentration. The SNP root ethanolic extract showed highest GDRI% of 78.33% at 30 mins, which may be due to its glucose adsorption ability of root extract that showed 45 mM.

The synthesis of SNPs in presence of ethanolic leaf and root extract showed 1ml to be potent under sunlight irradiation and room temperature respectively. The characterization studies such as UV spectrophotometer, FTIR, FESEM, EDS, and XRD confirmed the synthesis, size, shape and crystalline nature of SNPs. The average size for SNP leaf was 60.67 nm and 32.75 nm for SNP root with spherical form.

The GC-MS analysis showed that ethanolic leaf and root extracts of *C. anisata* contained a variety of bioactive compounds. The main constituent was 19-seco-15α-yohimban-19 oic acid, 20, 21-didehydro-16α-(hydroxymethyl)-, methyl ester and corynan-17ol, 18, 19-didehydro-10-methoxy- in leaf and root respectively. The alkaloid positive fraction of leaf and root extract obtained by column chromatography was subjected to *in vivo* studies.
The hypoglycemic studies under *in vivo* using alloxan-induced diabetic rats showed reduction in body weight, liver weight, pancreas weight, protein, plasma insulin, and reduced glutathione, glucokinase in liver and pancreas in diabetic control at 30\(^{th}\) day. The level of glucose, triglycerides, cholesterol, AST, ALT, ALP, fructose-1, 6-bisphosphate, glucose-6-phosphatase, LPO and SOD was found to be drastically increased in diabetic control at 30\(^{th}\) day. The SNP root (10 mg/kg bw) and glibenclamide (1 mg/kg bw) treated rats showed drastic increase in body weight with 138±0.26 g and 139.15±0.26 g respectively.

Comparatively, the SNP root (10 mg/kg bw) showed maximum increase in all the parameters that have been studied. In alloxan induced diabetic rats treated with SNP root (10 mg/kg bw) showed increase in liver and pancreas weight with 4.20±0.02 g and 1.48±0.02 g respectively. The glucose level was significantly reduced from 3\(^{rd}\) day to 30\(^{th}\) day in SNP root (10 mg/kg bw) treated diabetic rats with 133.75±0.37 mg/dl on 30\(^{th}\) day compared to control rats 103.77±0.60 mg/dl. The SNP root (10 mg/kg bw) showed major rise in protein level with 6.39±0.02 mg/dl and significant decrease in TG and cholesterol level with 93.67±0.33 mg/dl and 123.50±0.43 mg/dl respectively as compared with control.

The level of AST, ALT and ALP was found to be significantly decreased in SNP root (10 mg/kg bw) treated rats which is quite similar with control rats. The AST, ALT and ALP levels at 30\(^{th}\) day was found to be 32.17±0.31, 16.17±0.31 and 32.00±0.26 U/L, which is similar to standard drug glibenclamide (1 mg/kg bw) and control groups. The SNP root treated rats (10 mg/kg bw) showed significant decrease in fructose-1, 6-bisphosphatase and
glucose-6-phosphatase next to standard drug glibenclamide (1 mg/kg bw). The fructose-1, 6-bisphosphatase and glucose-6-phosphatase levels were 52.08±1.42 and 28.82±0.35 µM of Pi liberated/min/mg of protein on 30th day. Similarly, the glucokinase activity in liver and pancreas also increased on 30th day in SNP root (10 mg.kg bw) treated rats with 119.35±1.57 and 106.15±1.57 µM of glucose utilized/min/mg protein when compared with normal control 127.60±1.10 and 101.20±1.10 respectively.

LPO and antioxidants (reduced glutathione and SOD) were assayed. The level of these antioxidants was found to be potent in SNP root treated rats (10 mg/kg bw) that showed similar effect when compared with standard drug glibenclamide (1 mg/kg bw). The levels were 16.5±0.1 nmol MDA/g tissue, 13.1±0.2 µg/g tissue and 9.5±0.1 U/mg protein for LPO, reduced glutathione and SOD respectively. The SNP root extract at a concentration of 10mg/kgbw showed maximum increase of 6.36 ± 0.2516 µU/ml in plasma insulin level when compared with control (7.2 ± 0.2645 µU/ml) and standard drug glibenclamide (6.9 ± 0.20 µU/ml).

The Histopathological studies on pancreatic tissue revealed better restoration and maximum rise in beta cells in SNP root extract (10 mg/kg bw) treated rats as compared with standard drug glibenclamide. The present study disclosed that among the different extracts used the SNP root extract and glibenclamide significantly reversed the alloxan induced diabetic changes in rats. Interestingly, SNP root extract showed promising effect on hypoglycemic activity than other extracts, though it exhibited vital antioxidant, antibacterial and hypoglycemic activity in both in vitro and in vivo systems.
In conclusion, on the result of the present study among all extracts employed the SNP synthesized using the root extract of *C. anisata* showed maximum potent of antioxidant, antibacterial and hypoglycemic activities in both *in vitro* and *in vivo* conditions that may be due to the active phytoconstituents which was responsible for the activities. The extracts also inhibited the alpha amylase activity which may results in delayed digestion of the dietary carbohydrates, thereby lowering the glucose liberation. The liberated glucose absorption and diffusion into circulation from the lumen is also inhibited. The maximum *in vitro* hypoglycemic activity of SNP root extracts was intervened by decreasing the glucose diffusion rate, increasing the glucose adsorption rate and by glucose transport across the cell membrane. This is illustrated by its action against the *in vivo* model, implying its potential to treat type II diabetes. The SNPs synthesized from *C. anisata* ethanolic extract was characterized by UV-Vis, FTIR, XRD, EDS and FESEM that supports the stability of SNPs. The extracts may be acted as a reducing and capping agent. The antibacterial activity of SNP extract showed wider activity against gram negative than gram positive organisms. The crude ethanolic and SNP root extracts comparatively showed powerful effects close to the positive controls used in the respective studies. Results of this study showed that SNP root extract, has effective hypoglycemic activity against alloxan induced diabetic rats, by reducing blood glucose level, triglycerides, cholesterol, AST, ALT, ALP, fructose-1, 6-bisphosphatase, glucose-6-phosphatase, LPO and SOD and increasing body weight, reduced glutathione, the activity of glucokinase in liver and pancreas, protein level, liver and pancreas weight. The histopathological sections of pancreas of alloxan induced diabetic rats
showed a reduction in size of islets when compared to that of normal group. The crude root extract and SNP root extract has restored the size of islets along with beta cell repair. The recovery of beta cell was found to be dose dependent. On the other hand, some extracts showed significant, moderate and weaker activities compared to the standard in alpha amylase inhibition assay and glucose uptake by yeast cells. The phytoconstituents of *C. anisata* extracts may be acting synergistically with antioxidant properties along with hypoglycemic effects in exerting an overall antidiabetic action in this study, and that should be chemically analyzed and their chemical structure should be understood in order to develop an effective diabetic therapeutic agent in future.