

# Antioxidant and Hypoglycemic Activities of *Clausena anisata* (Willd.) Hook F. Ex Benth. Root Mediated Synthesized Silver Nanoparticles

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## ABSTRACT

The present study was carried out to examine the hypoglycemic effect of Silver Nanoparticles (SNPs) using ethanolic root extract of *Clausena anisata* (Willd.) Hook. f. ex Benth. A cost effective and eco friendly technique for green synthesis of SNPs from 1mM AgNO<sub>3</sub> solution through the root extract was carried out. The biosynthesized SNPs were characterized using UV spectrophotometry, Field Emission Scanning Electron Microscopy (FESEM), X-ray Diffraction analysis (XRD), Energy Dispersive Spectroscopy (EDS) and Fourier transform infra-red (FTIR) spectrometry. DPPH assay was done to determine the antioxidant activity and for *in vitro* hypoglycemic activity glucose uptake by yeast cells, alpha amylase inhibition assay, adsorption capacity and glucose diffusion assay was performed. The SNPs of ethanolic root extract were found to be spherical in shape and up to 32.75 nm average in size. They showed alpha amylase inhibitory activity of 83.60% at 500 µg/ml. The glucose uptake by yeast cells was found to increase with an increase in concentration. The maximum glucose uptake was found to be 69.51% at 10mM concentration. The molar concentration of glucose was directly proportional to the glucose binding capacity of extracts. The rate of glucose diffusion across the membrane was found to increase from 30 to 180 minutes. The DPPH scavenging activity was found to be potent (74.07%) at 500 µg/ml. The hypoglycemic effect exhibited by the SNPs was revealed by simple *in vitro* model of yeast cells, mediated by glucose adsorption, increasing glucose diffusion and glucose transport across the cell membrane.

**Key words:** *C. anisata*, Silvernanoparticles, Diabetes, Antioxidant activity, Glucose and Silver nitrate.

## INTRODUCTION

Diabetes Mellitus is a multifactorial metabolic disease characterized by hyperglycemia, including abnormal carbohydrate, fat and protein metabolism leading to several complications and hence needs a combined therapeutic approach.<sup>1</sup> Insulin injections are given in case of total lack of insulin; the post prandial hyperglycemia is managed at digestive level by using acarbose, miglitol and voglibose drugs.<sup>2</sup> In India, the number of people affected with diabetes is expected to rise from 171 million in 2000 to 366 million by 2030.<sup>3</sup> Out of the two major types of diabetes, type II diabetes is more prevalent and mainly due to the loss of pancreatic β-cell function, which results in insulin resistance. The regeneration or stabilization of β-cell must occur, in order to prevent the loss of β-cells.<sup>4</sup>

Even though there are several drugs to tightly regulate blood glucose, to reduce microvascular and macrovascular complications, the main undesirable effects of this anti-diabetic drug that are currently available are brain damage, swelling, erythema, abdominal

pain, weight gain, metallic taste, vitamin B<sub>12</sub> deficiency, heart failure and gastro intestinal disturbances. Due to these side effects of oral hypoglycemic agents and oxidative stress in complicating diabetes,<sup>5</sup> there is growing interest in herbal remedy for the treatment of type II diabetes mellitus and oxidative stress.

In India, more than 150 plants of different families were reported to have hypoglycemic activity. Among them, *Clausena anisata* (Willd.) Hook f. ex. Benth, is one which belongs to the family *Rutaceae*,<sup>6</sup> is represented by about 20 species in India and are used traditionally for the treatment of several ailments such as rheumatism, cold, arthritis, analgesic, heart disorder, hepatic disease, antiseptic, anthelmintic, gastro intestinal disorders, fever, headache, sinusitis, wounds, sprains, fractures, toothache, mouth infection, stimulate insulin secretion<sup>7</sup> and convulsions.<sup>8</sup> As per traditional claims, *Clausena anisata* was reported to possess insulin stimulating activity. Even though the plant is explored for antidiabetic activity

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but no reports are available on the mechanisms of their hypoglycemic effects. Hence based on the traditional indication, the present study was aimed to evaluate their effects on glucose adsorption, diffusion and glucose uptake by yeast cells using *in vitro* techniques that will elucidate the plausible mechanism of its activity. The antioxidant and hypoglycemic activity of ethanolic SNP root extracts of *C. anisata* has not been reported till date under *in vitro* conditions. Evaluating their potential will provide an effective utilization as therapeutic agents for Type II diabetes.

## MATERIALS AND METHODS

### Procurement of plant material

Fresh roots of *Clausena anisata* (Figure 1) were procured during the months of October and November (2013) from Manamettupatti of Viralimalai Taluk (Pudukottai District, Tamil nadu). The plant was identified by Rev. Fr. Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics of St. Joseph's College, Tiruchirappalli-620 002 and authenticated as *Clausena anisata* (Willd.) Hook. f. ex Benth. Vouchered herbarium by specimen No: LB NSD 001.

### Preparation of plant extract and SNPs

10g of shade dried root powder was macerated at 35°C to 37°C; three to five times for 15–20 hrs with 100 ml of ethanol in an Erlenmeyer flask (500 ml). Then the extract was centrifuged, filtered using Whatmann No. 1 filter paper and safely stored. The biosynthesis of SNP was carried out as outlined by the method.<sup>9</sup>

### Optimization and synthesis of SNPs

1ml, 3ml and 5 ml of ethanolic root extracts were taken in a separate conical flask and to this 10 ml of 1 mM AgNO<sub>3</sub> solution was added with constant stirring and were exposed under different conditions like sunlight radiation, direct boiling, microwave irradiation (10s on, 10s off) to prevent overheating and aggregation of metals, UV irradiation and room temperature (Figure 2). The color change of the root extract was checked periodically and the color changes from beige to dark brown indicate the synthesis of SNPs from the roots.

### Production and recovery of SNPs

For bulk production, 10 ml of root extract in 100 ml of 1 mM AgNO<sub>3</sub> was prepared. After bio reduction, the extract consisting of SNPs was subjected to centrifugation at 10,000 rpm for 20 min, and the supernatant was discarded. To the pellet, 0.1 ml of toluene water was added, air dried, lyophilized and stored for further characterization.

## CHARACTERIZATION STUDIES

The biosynthesized SNPs were characterized by the following methods:

### Visual Observation

A change of color from beige to reddish brown was periodically noticed in the root extract exposed to different conditions as illustrated earlier.

### UV–Vis Spectrophotometric analysis

The formation of SNPs was confirmed and was studied using the spectral analysis. The UV spectra of the biosynthesized SNPs were recorded using Lambda 35, Perkin Elmer Spectrophotometer by continuous scanning from 190nm to 1100nm and distilled water was used as the reference.

### Fourier Transform Infra Red Spectroscopy Analysis (FTIR)

The functional groups in the biosynthesized SNPs were analyzed by FTIR spectroscopy. These measurements were carried using a Perkin Elmer

spectrum RX I FTIR instrument with a wavelength range of 4000cm<sup>-1</sup> to 400 cm<sup>-1</sup>. The results were compared for shift in functional peaks.

### Field Emission Scanning Electron Microscopy (FESEM)

FESEM was used to characterize the mean particle size, morphology of the SNPs. A small drop of biosynthesized SNPs solution was placed on glass slide and allowed to dry. The samples were analyzed by using FEI Quanta 200 FEG machine at a low vacuum in the range 10 – 20Kv.

### Energy Dispersive Spectroscopy (EDS)

The elemental composition of the synthesized SNPs was analyzed with energy dispersive spectroscopy coupled to scanning electron microscope.

### X-ray Diffraction Analysis (XRD)

The structure and composition of SNP were studied by XRD (XPRT-PRO Machine). The data was collected in the 2θ range.

### Determination of antioxidant activity (DPPH assay)

The antioxidant activity of the SNPs was evaluated by DPPH radical scavenging assay.<sup>10</sup> SNPs of different concentration ranging from 100 µg/ml - 500 µg/ml were prepared. To this, DPPH at a concentration of 0.1mM was added and incubated at room temperature for 30 min. The absorbance was recorded at 517 nm. The control sample was carried out without adding leaf extract. Ascorbic acid was used as a standard control. The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test sample}}}{\text{Abs}_{\text{control}}} \times 100$$

### *In vitro* Hypoglycemic Activity

#### Alpha–amylase inhibition assay

The alpha-amylase inhibitory activity was carried out using a carbose as the reference compound based on the spectro photometric assay.<sup>11</sup> The SNPs at a concentration of 100 - 500 µg/ml was prepared by dissolving in DMSO. 3.246 mg of α-amylase(0.5 U/ml) (EC 3.2.1.1) was dissolved in 100 ml of 20 mM phosphate buffer (pH 6.7) containing 6.5 mM sodium chloride. Seven duplicate test samples including the blank and control were prepared. In each sample, 250 µl of the enzyme was added with 100 µl of SNP extracts of different concentrations except blank and were incubated in a water bath at 37°C for 20 min with constant stirring in a vortex. After incubation, 250 µl of starch (0.5% w/v starch dissolved in 20 mM phosphate buffer, pH 6.7) was transferred into each sample in the test tube to initiate the reaction. It was again incubated at 37°C for 15 min after vortexing. DNS reagent of 2 ml was added and stirred in a vortex. Later it was boiled in water bath at 100°C for 10 min, thereafter the mixture was cooled down and the absorbance was measured at 540 nm and control sample was carried out without adding the leaf extract.

Percentage inhibition was calculated by the expression:

$$\% \text{ Inhibition} = 100 - \frac{\text{Mean product in sample}}{\text{Mean product in control}} \times 100$$

#### Glucose uptake by yeast cells

Commercial baker's yeast was prepared<sup>12</sup> in distilled water by repeated centrifugation at 21000 rpm for 5 mins until the supernatant was clear. The 10% (v/v) concentration of yeast suspension was prepared in distilled

water. SNP extracts at concentration of 100 µg-2 mg were added separately to 1 ml of glucose solution (5, 10, 25 mmol/L) and incubated for 10 minutes at 37°C. To this, 100 µL of yeast suspension was added to start the reaction and vortexed. Then it was incubated at 37°C for 60 min. After 60 min, at 3800 r/min for 5 min the tubes were centrifuged and the glucose present in the supernatant was estimated at 540 nm.<sup>13</sup>

The percentage of glucose uptake by yeast cells was calculated using the formula:

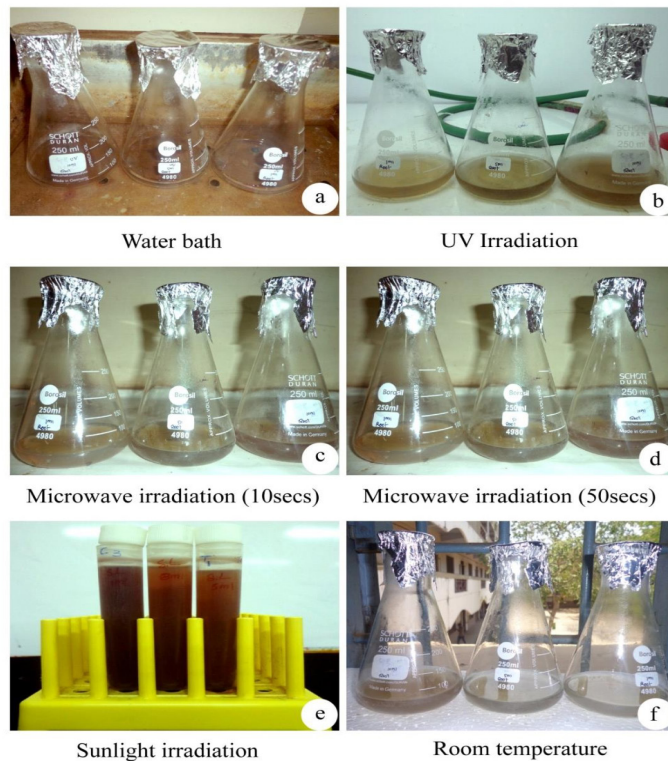
$$\text{Percent increase in glucose uptake (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, Abs control and Abs sample is the absorbance of the control reaction (without the addition of SNPs) and test sample respectively.

### Glucose Diffusion Assay

1 mL of glucose solution at a concentration of 25 mmol/L, was prepared by dissolving in 0.15 M NaCl<sup>14</sup> and 1% of SNPs were dialyzed in two separate dialysis bags for control and test sample. Then the dialysis membrane was tied at both ends and immersed in a separate beaker containing 10 ml of distilled water and 40 ml 0.15 M NaCl. For control, SNP extract was not added. The beakers were incubated at room temperature. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min by DNS method.<sup>15</sup> Glucose Dialysis Retardation Index (GDRI) was calculated by using the formula:

$$\text{GDRI (\%)} = 100 - \frac{\text{Glucose content (with sample mg/dL)}}{\text{Glucose content (without sample mg/dL)}} \times 100$$



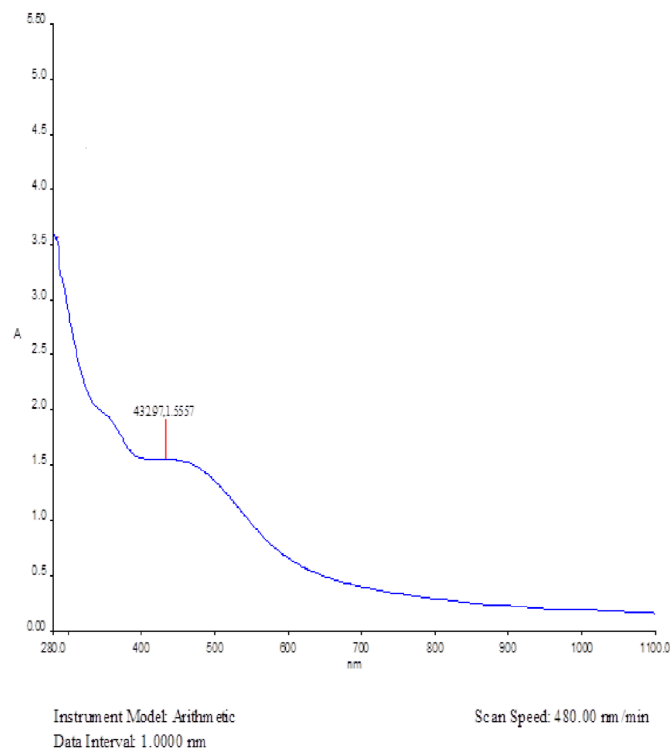
**Figure 2:** Optimization and synthesis of SNP using *C. anisata* ethanolic root extract.



**Figure 1:** Roots of *C. anisata* collected from Manamettupatti.

### Dinitrosalicylic colorimetric method (DNS)

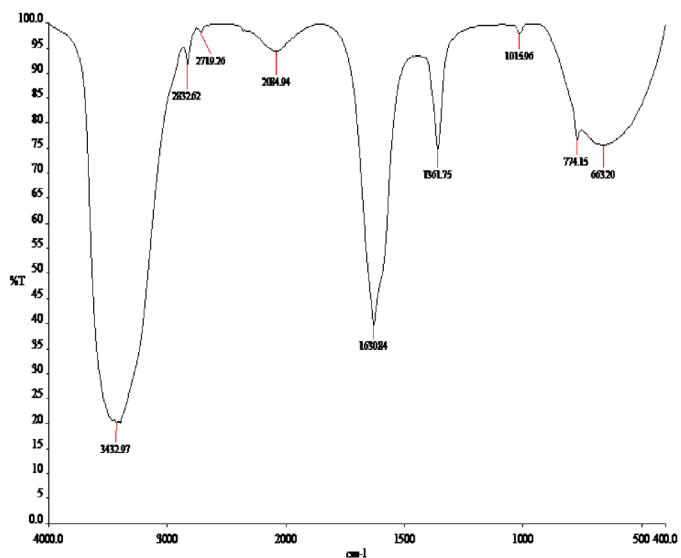
After incubation, 250 µl of the starch (0.5% w/v of starch was dissolved in 20 mM phosphate buffer (pH 6.7)) was transferred into each test tube carrying glucose content of dialysate at 30, 60, 120 and 180 mins. The vortexed mixture was incubated at 37°C for 15 mins. DNS reagent of 2 ml was added, stirred in a vortex and boiled in water bath at 100°C for 10 mins. The mixture was cooled and the absorbance was measured at 540 nm.



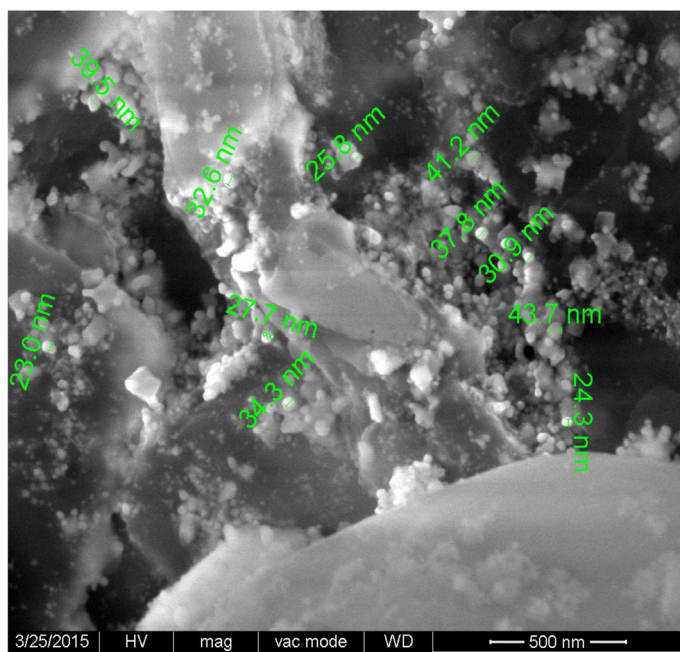
**Figure 3:** UV-Vis profile for ethanolic SNP root extracts of *C. anisata*.

**Table 1: FTIR spectral peak values and functional groups obtained for the SNP root extract of *Clausena anisata***

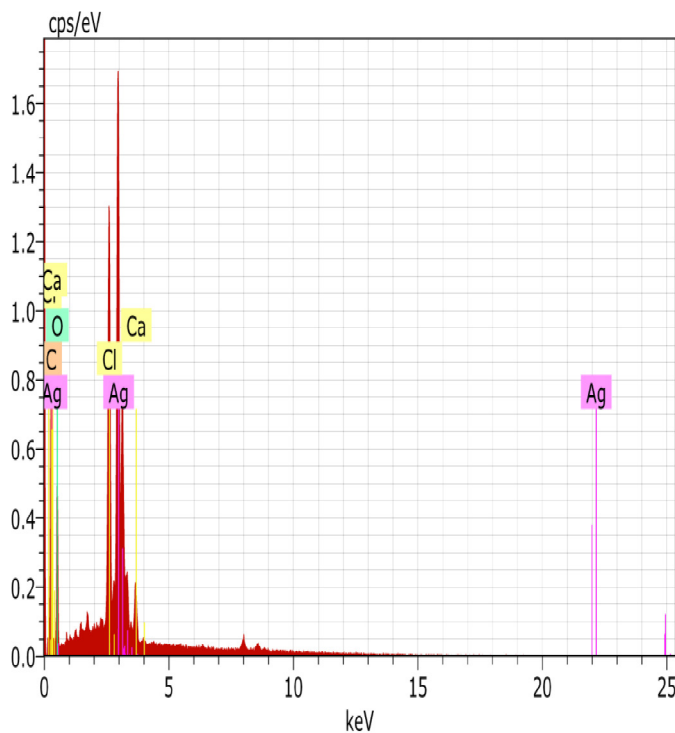
S.No	Peak value $\text{cm}^{-1}$	Bond	Functional groups
1	663.20	C-H bend	Alkynes
2	774.15	C-Cl Stretch	Alkyl halide
3	1015.96	C-O stretch	Alcohol
4	1361.75	N-O symmetric stretch	Nitro compounds
5	1630.84	$\text{RNH}_2$	Amines
7	2832.62	H-C=O	Aldehyde
8	3432.97	N-H stretch	Amine, amide



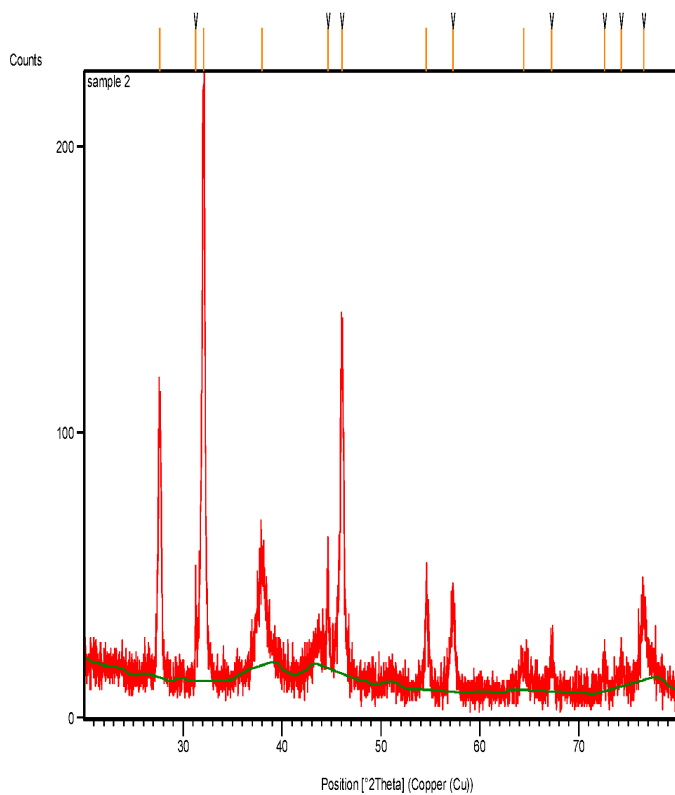
**Figure 4:** FTIR spectrum of *C. anisata* ethanolic SNP root extracts.



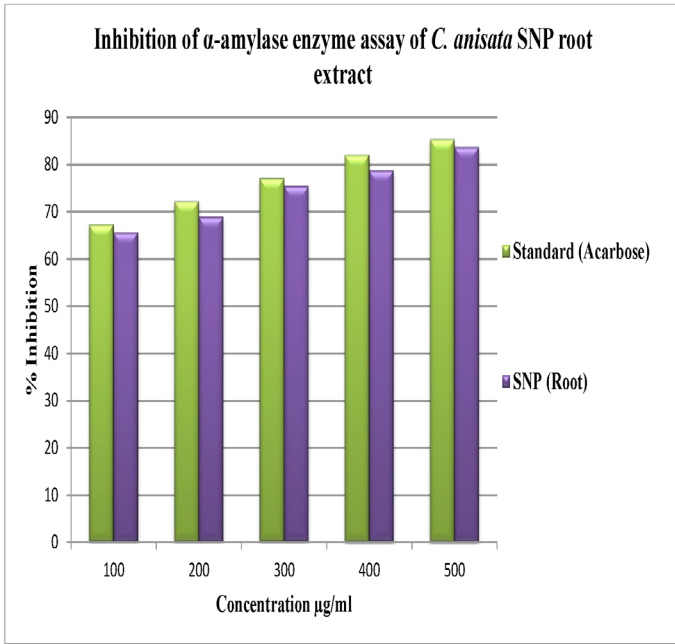
**Figure 5:** SEM analysis of *C. anisata* SNP root extract.



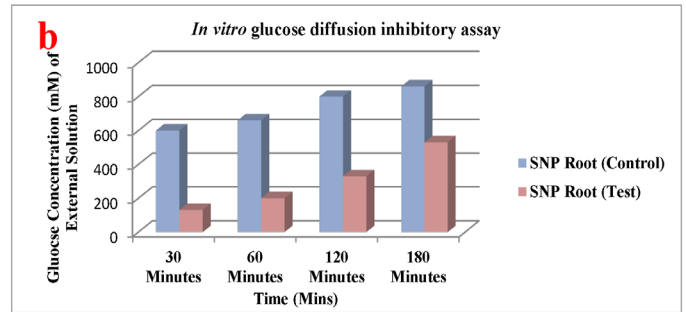
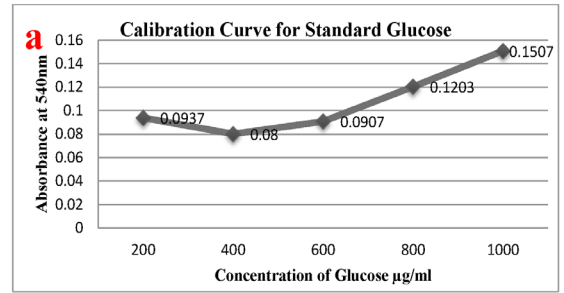
**Figure 6:** EDS analysis of ethanolic root extract of *C. anisata*.



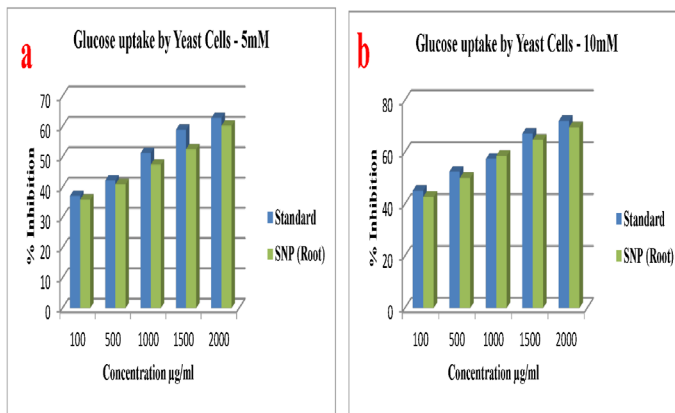
**Figure 7:** XRD analysis of *C. anisata* SNP root extract.



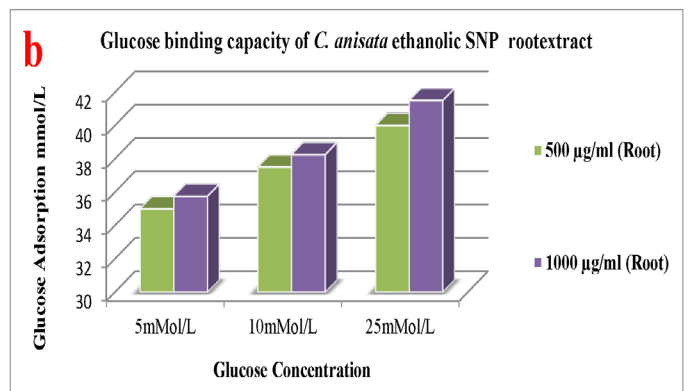
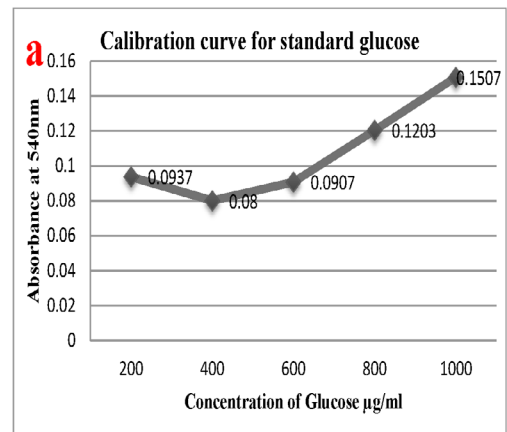
**Figure 8:** Percentage inhibition of  $\alpha$ -amylase enzyme assay of *C. anisata* SNP root extract.



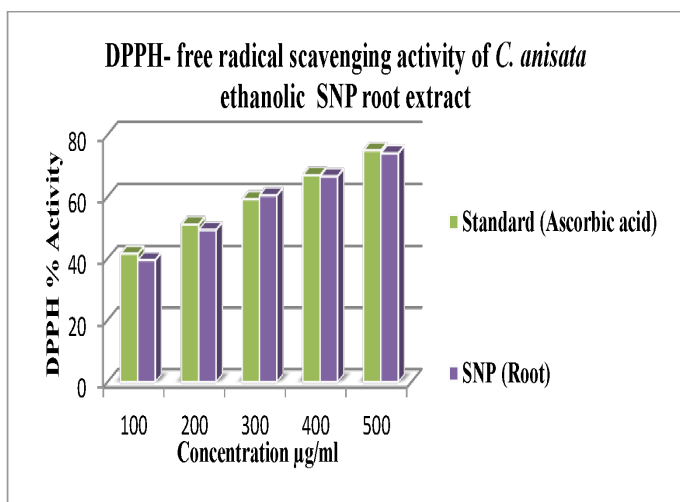
**Figure 10:** a) Standard calibration curve for glucose estimation b) Glucose concentration in the dialysate in presence of *C. anisata* extracts.



**Figure 9:** Glucose uptake by yeast cells in presence of *C. anisata* SNP root extract a) 5 mM b) 10 mM c) 25 mM.



**Figure 11:** Standard calibration curve for glucose estimation b) SNP root extract.



**Figure 12:** DPPH percentage scavenging activity of *C. anisata* SNP root extracts.

### Glucose Adsorption Capacity

SNP at a concentration of 1% was added to 25 mL of 5, 10, and 25 mmol/L glucose solution in a four separate experiments. The content was vortexed and incubated in a water bath at 37°C for 6hrs and centrifuged at 4800 rpm/min for 20 mins.<sup>16</sup> The glucose content in the supernatant was determined at 540 nm. The bound glucose concentration was calculated using the formula:

$$\text{Bound glucose concentration} = \frac{G_1 - G_6}{\text{Wt. of the sample}} \times \text{Volume of Solution}$$

$G_1$  is the glucose concentration at 0<sup>th</sup> hour

$G_6$  is the glucose concentration after 6 hours

### Statistical analysis

The results were expressed as the mean  $\pm$  standard deviation from three independent experiments with triplicates. One way analysis of variance (ANOVA) was performed followed by Tukey's test.  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

Among various concentration and methods used, 1ml of extract exposed under room temperature was found to be very effective and showed maximum synthesis of nanoparticles. In this study it was observed that there was visible color change when silver nitrate was added to the root extract. Initially the leaf extract was beige in color and after the addition of silver nitrate; it was turned into brown in colour (Figure 2e). The ethanolic root extract of *C. anisata* reduced silver ions into SNP was evidenced by visual colour change. This indicated the formation of SNPs due to excitation of surface Plasmon vibration in silver nanoparticles.

### UV – Vis spectral analysis of SNPs

The color change of root ethanolic extracts of *C. anisata* arose due to the excitation of SPR with the SNPs. The SPR of SNPs produced a peak centred near to 432.97 nm (Figure 3) for root, with absorption 1.5557 respectively. These results indicated the successful conversion of silver nitrate ( $\text{Ag}^+$ ) to Silver ( $\text{Ag}^0$ ). The SPR band is due to the free electrons in the conduction band due to small particle size.<sup>17</sup> Due to the combination of proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins,

phenolics, saponins and terpenoids may be responsible for the reduction and stabilization of silver ions.<sup>18</sup> Proteins that have stronger binding affinity to SNPs will increase the stability of synthesized NPs.<sup>19</sup>

The absorbance peaks obtained for *C. anisata* root extracts was in line with<sup>20</sup> 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.

### FTIR Spectrum

FTIR analysis was used to identify the functional groups of the active components in the extracts that are responsible for reducing and capping the synthesized SNPs. Eight IR bands were identified for SNP root extracts which was tabulated. The absorption peak of  $1630.84 \text{ cm}^{-1}$  for SNP root in the infrared region of the electromagnetic spectrum exhibited the binding of amide linkage with SNPs that may be assigned to the carbonyl stretch in proteins and clearly indicated the presence of protein as capping agent for SNPs. FTIR spectral data for the biosynthesized SNP root extract were attributed to the N-H stretch at  $3432.97 \text{ cm}^{-1}$ , H-C=O stretch exists in the region of  $2832.62 \text{ cm}^{-1}$ , peaks at  $663.20 \text{ cm}^{-1}$ ,  $774.15 \text{ cm}^{-1}$ ,  $1015.96 \text{ cm}^{-1}$ ,  $1361.75 \text{ cm}^{-1}$  and  $1630.84 \text{ cm}^{-1}$  can be assigned to C-H bend, C-Cl stretch, C-O stretch, N-O symmetric stretch and  $\text{RNH}_2$  stretch (Figure 4). 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 alkynes bonds and responsible for compounds like alkaloid, flavonoid and terpenoids that may be act as capping and stabilizing agent for SNPs.<sup>20,21</sup>

### FESEM image analysis

This analysis was carried out to measure the size and shape of the SNPs. The synthesized SNPs showed spherical form and with a size distribution ranging from 23 nm to 44 nm, with average size 32.75 nm was found to be for SNP root (Figure 5). The SEM results are also consistent with those of<sup>22</sup> who reported that synthesis of SNPs from aqueous leaf extract of *Phyllanthus amarus* showed particle ranging between 32-53 nm.

### EDS Analysis

The EDS analysis was performed to know the percentage of silver in the sample at 3KeV. The EDS spectra showed the different types of elements with their weight percentage such as silver (24.72%), carbon (38.78%), oxygen (28.25%), chlorine (6.37%) and calcium (1.88%) respectively (Figure 6). In this study, the other weak signal corresponding to  $\text{O}_2$ , 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.<sup>23</sup>

### XRD Characterization

The XRD spectra were used to confirm the crystalline nature of the SNPs. The X-ray diffraction pattern of SNP was confirmed by the characteristic peak observed in XRD image. The biosynthesized SNPs by using extracts of *C. anisata* was further confirmed by the characteristic peaks observed in the XRD image at  $2\theta$  angles with minimum and maximum range at  $27.64^\circ$  -  $76.53^\circ$  for SNP root (Figure 7). Here, the several Bragg's reflection peak was pointed towards crystal structure of silver. The few unassigned Bragg's peak might be due to capping agent stabilizing the NPs with were consistent with the earlier report.<sup>24</sup>

### Alpha-Amylase inhibition assay

The SNP root extract exhibited 83.60% ( $P < 0.001$ ) inhibition respectively at 500  $\mu\text{g/ml}$ , in which the standard percent inhibition was found to be

85.24% (Figure 8). The results obtained for SNP extracts of 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  $\mu\text{g/ml}$ , where a carbose showed 513.97  $\mu\text{g/ml}$ .<sup>25</sup>

The hallmark of DM is hyperglycemia, produced by glucose after few minutes of ingestion. The carbohydrate hydrolyzing enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) are responsible for breaking  $\alpha$ -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 ( $\text{Na}^{2+}$ ).<sup>26</sup> 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.<sup>27,28</sup>

### Glucose uptake by yeast cells

**Inhibition of glucose uptake in 5mM, 10mM and 25mM glucose concentration:** Glucose transport across the yeast cell membrane was studied in an *in vitro* system comprising of yeast cells along with varying concentrations of glucose solution in the presence of the plant extracts. The indicator of the glucose uptake by yeast cells was the amount of glucose remained in the medium after a specific time. The glucose uptake for SNP root was found to be between 35% to 61% in 5 mM of glucose, 42% to 70% in 10 mM of glucose and 44% to 64% in 25 mM of glucose concentration. The SNP extracts of root showed maximum uptake of glucose by yeast cells at 10 mM glucose concentration with 69.51% ( $P < 0.001$ ) (Figure 9). These extracts also showed non-linear increase in glucose uptake. Further studies are needed to validate these variations in glucose uptake by yeast cells. The significant uptake for SNP root was at 1000  $\mu\text{g/ml}$  ( $P < 0.0001$ ) in 10 mM glucose concentration.

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  $\mu\text{g/ml}$ .<sup>29,30</sup> This may also be due to the inhibition of enzyme  $\alpha$ -amylase, which inhibits the release of glucose from starch, due to the high concentration of fiber, viscous polysaccharides, encapsulation of enzyme and starch that reduce its accessibility.<sup>30</sup>

### Glucose Diffusion Inhibitory Assay

GDRI, an *in vitro* index was used to predict the effect of fibers present in the extract, which is responsible to delay the glucose absorption in gastro intestinal tract, was calculated and the results obtained were shown. The glucose movement was monitored from 30 mins to 180 mins. The rate of glucose in the dialysate was measured using standard calibration curve for known concentration of glucose. The higher GDRI index was found in the ethanolic extracts of SNP root (78.33%) ( $P < 0.001$ ) at 30 min. 1 ml of sample from dialysate was collected in intervals and glucose estimation was done by DNS method. The concentration of glucose ranges from 100  $\mu\text{g}$  to 600  $\mu\text{g/ml}$  (Figure 10). This results show that the concentration of glucose inside the dialysis membrane gradually decreased due to the adsorption ability of plant extracts.

### Glucose Adsorption Capacity

The glucose adsorption capacity of SNP root extracts of *C. anisata* at different glucose concentration (5 mM, 10 mM & 25 mM) was investigated in this study and the results were presented in the.

The adsorption capacity of the extracts was found to be directly proportional to the glucose molar concentration and the glucose binding capacity was found to increase with increased molar concentration of glucose

(5 mM, 10 mM and 25 mM). The rate of glucose adsorbed was calculated using standard calibration curve for known concentration of glucose (Figure 11). The SNP root extract showed 41.5 mMol/L ( $P < 0.0001$ ) at 1000 $\mu\text{g/ml}$ . From this result it is clear that as the concentration of extract increases, the glucose adsorption capacity also increases. The dietary fibers have the ability to bind glucose and showed adsorption capacity for glucose, only when the concentration of glucose increases.<sup>31</sup> 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.

### Determination of antioxidant activity (DPPH assay)

A simple reliable DPPH method was carried out to evaluate the antioxidant activity of different leaf, root, SNP leaf and SNP root extracts of *C. anisata*. The scavenging activity of the plant extracts through the annihilation of the DPPH radicals was tabulated. The SNP root extract showed maximum activity of 74.07% and standard showed 75% inhibition of scavenging activity (Figure 12). When the free radical concentration exceeds the level of antioxidant mechanism it leads to various diseases including diabetes. 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.<sup>32,33</sup>

### CONCLUSION

Based on the result of the present study, the SNP synthesized using the root extract of *C. anisata* showed maximum potent of antioxidant, antibacterial and hypoglycemic activities in *in vitro* condition that may be due to the active phytoconstituents which was responsible for the activities. The extract 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. 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.

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### CONFLICT OF INTERESTS

All authors disclose no conflict of interest

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