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ANTIOXIDANT, ANTIDIABETIC AND ANTIPERIODONTAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES USING FLOWER AND STEM OF *COSTUS IGNEUS*

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ABSTRACT

In the present study, explains the detailed information about the synthesis of sliver nanoparticles using the flower and stem of *Costus igneus* which was used to determine the alpha amylase, alpha glucosidase inhibition assay and antioxidant activities are examined by DPPH (diphenyl-2 picrylhydrozyl) method. The characterization of synthesized silver nanoparticleswere confirmed by the UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy dispersive X-ray (EDX). The UV- visible spectroscopy was used to examine the silver nanoparticles and the peak range was observed at 436nm. The FTIR was used to analyses the functional group present in the plant extracts and silver nanoparticles. The SEM analysis showed the cubic and spherical shape of the synthesized silver nanoparticles. The elemental composition of the synthesized silver nanoparticles is determined by EDX analysis. The crystalline nature of the silver nanoparticles was analyzed by using XRD technique. The result shows that the flower-AgNPs exhibits inhibitory effect on alpha amylase (84.9%) and for the stem-AgNPs (82.5%). alpha glucosidase showed inhibitory effects in comparison with flower-AgNPs (58.3%) and stem-AgNPs (53.7%) by using standard acarbose drug. The antioxidant activities by DPPH method exposed activity for flower-AgNPs (81.3%) and for the stem-AgNPs (79.6%) were compared with standard ascorbic acid. The antiperiodontal analysis showed a better inhibition against *streptococcus mutans, streptococcus salivaryus*,

streptococcus sobrinus and *staphylococcus aureus*. Therefore, it is suggested that the synthesized silver nanoparticles using flower and stem of *Costus igneus* extract as the good yield of inhibitor for diabetes and periodontitis.

Keywords: Flower and stem-AgNPs of Costus igneus alpha amylase and alpha glucosidase inhibitory activity, antioxidant (DPPH) activity, antiperiodontal activity

1. INTRODUCTION

Diabetes mellitus is a developed by the body cells cannot properly metabolise the sugar due to lack of insulin secretion and insulin action **[1]**. Type1 diabetes mellitus is also called as juvenile diabetes in this condition in which leads to autoimmune destruction of insulin producing β-cells in the pancreas **[2]**. Type 2 DM is occur due to obesity and is characterised by an initial phase of progressive insulin resistance, with an ensuring reduction in the ability of pancreatic hormone to promote glucose disposal and to suppress hepatic glucose output **[3]**. Periodontitis is the sixth complication of diabetes. Neutrophils impairment leads to increased susceptibility to periodontitis in diabetic **[4]**. Diabetes prolongs the inflammatory response to *porphyromonas gingivalis* with increased production of TNFα **[5]**. The word 'periodontitis' comes from peri (around), odont (tooth) and it is (redness). Periodontitis is a more severe inflammation because not only it affects the tissue but also affects the bottom of the teeth. It is not treated at all it may cause loss of teeth **[6]**. *Costus igneus* is also called as insulin plant which belongs to the family costaceae. The flower and stem parts of the plants contains protein, iron and antioxidant which includes ascorbic acid, α-tacopherol, β-carotene, terpenoids, steroids, and flavonoids **[7]**. The anti-inflammatory activity result in isolated compound β-amyrin has shown 97% inhibition of paw edema at a given close of 100µg. The antibacterial and antimicrobial activity against for both gram positive and gram negative bacteria. The hypoglycaemic result in 7.570% reduction of blood glucose level **[8]**. This present study shows the effect of flower and stem extract of *Costus igneus* on Diabetes mellitus and periodontal disease.

2. MATERIALS AND METHODS:

2.1. Collection of Plant Sample:

The *Costus igneus* stem and flower were collected in Srirangam, Tiruchirappalli district, Tamil Nādu, India.

2.2. Preparation of plant Extract:

The collected stem and flower samples were thoroughly washed with tap water and air dried in a shadow that is free from sunlight till it becomes dried nicely. Then it is crushed in an blender and the powder was separated, which were stored individually in air-tight containers and kept

in a cool, dark and dry place for further study. The ethanol extract was prepared by taking 20g of the powdered sample and it is soaked in 50ml of ethanol for 24hours. Then it was extracted using hot percolation method **[9]**.

2.3. Chemical and Reagent:

Ammonium Hydroxide, Amyl Alcohol, sodium Hydroxide, Ascorbic Acid, conc.H2SO4, Chloroform, Ferric chloride, Acetic Acid, Benzo ethanol, Silver Nitrate, Ethanolic alpha Naphthol, PNPG, Sodium Carbonate DNS Diethyl Ether, petroleum Ether **[10]**.

2.4. Synthesis of Silver Nanoparticles:

5ml silver nitrate was prepared in a 100ml standard flask. 2.5ml of the sample is added to 50ml of 5mM silver nitrate with continuous and constant stirring which react at an ambient condition and Ag get reduced in to Ag+ ion. The colour change was observed for the reaction mixture from transparent white to dark brown indicates the formation of silver nitrate. The presence of reduction of Ag+ ion was confirmed overtime by the UV-Spectral analysis **[11, 12]**.

2.5. Phytochemical Screening:

The stem and flower of *Costus igneus* are used for the quantitative and qualitative analysis of phytochemicals screening. The result showed the presence of flavonoid, terpenoids, saponins, tannins, alkaloids, steroids, glycoside, phtobatannins, protein, coumarins, emodin, anthraquinone, anthocyanins, carbohydrates, leucoantiocyanin, Cardiacglycoside, xanthoprotein, phenol were present in phytochemicals screening test **[13]**.

2.6. Characterization of Synthesized Silver Nanoparticles:

2.6.1. UV-visible Spectrophotometry:

The extracts were examined under visible and UV light for proximate analysis. Each and every analysis was repeated twice for the spectrum confirmation. For UV and FTIR Spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using high pressure vacuum pump. The sample was diluted to 1:10 with the same solvent. The extracts we are scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected **[14]**.

2.6.2. FT-IR:

FT-IR analysis was performed to classify the biomolecules responsible for reduction of the metals and for the stabilization of nanoparticles the functional group AgNo3 analysed using FTIR RX1perkin Elmer in wavelength range 400-400cm-^{1}**[15]**

2.6.3. XRD:

X-ray based on construction of monochromatic x-rays and crystalline sample non- destructive techniques XRD

that provides information the crystallographic structure chemical and physical properties and materials **[16]**.

2.6.4. SEM:

SEM-Scanning electron microscope highly versatile methodologies for 20 and 30 materials characterization **[17]**.

2.6.5. EDX:

EDX particularly valuable in the research of drug. EDX is a crucial tool for nanoparticles used to improve the therapeutic of chemotherapeutic agents.

2.7. ANTIDIABETIC ACTIVITY:

2.7.1. Alpha Amylase Assay of Silver Nanoparticles Synthesized from *Costus Igneus* Stem and flower Extract [17]:

Into a series of test tube (20-100µg /ml) various concentration of $AgNo_3$ was taken and made up to 1ml with ethanol and mixed it well. Then 500µl of various concentration of stem and flower extract was taken in a test tube and add 250µl of α-amylase. This solution was incubated for 10minutes. Then add 250µl of 1% starch and incubated for 10 minutes. After the incubation is over add 500µl of DNS (Dintrosalicilic acid). Incubated the solution into the water bath for 5minutes. After add 5ml of distilled water. The spectrophotometer was used to measure the absorbance at 540nm. The α-amylase inhibitory activity was calculated in terms of percentage inhibition as follows

%inhibition = [(Abs control –Abs synthesized nanoparticles/Abs control)]× 100

A and B represents the absorbance value for the test and blank sample. The %inhibition versus concentration curve and 50% inhibition was determined in a sample graph is plotted.

2.7.2. Alpha Glucosidase Inhibition Assay of Silver Nanoparticles Using *Costus Igneus* Stem and flower Extract:

Into a series of test tube (20-100µg /ml) various concentration of $AgNo_3$ was taken and made up to 1ml with ethanol and mixed it well. Then 500µl of various concentration of extract was taken in a test tube and add 250µl of α-glucosidase enzyme. This solution was incubated for 10minutes. To this solution add 250µl of PGNP (4-nitrophenol β-D glucopyranoside) and incubated for 10 minutes. This solution was changed into yellow colour. After that add 2ml sodium carbonate and incubated at 5minutes. The spectrophotometer was used to measure the absorbance at 540nm. The α-glucosidase inhibitory activity was calculated in terms of percentage inhibition as follows

%inhibition = [(Abs control –Abs silver nanoparticles/Abs control)]× 100

A and B represents the absorbance value for the test and blank sample. The %inhibition versus concentration curve and 50%

inhibition was determined in a sample graph is plotted.

2.8. Collection of Test Pathogen

The anti-periodontal activity of synthesized ethanol extract from flower and stem extract of *Costus igneus* was shown against *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus mutan* were prepared as test organisms. All the bacterial strains were purchased from the Microbial type Culture and collection at Chandigarh, India. Determination of Anti-periodontal activity by Disc Diffusion Method: The disc diffusion method is used to determine the anti-periodontal activity of the synthesized nanoparticles using flower and stem extract of *Costus igneus*. The obtained compound was allowed to bind with the paper disc for sometimes. 25ml of Muller-Hilton agar medium was poured into sterile petri dishes and inoculated with test organisms in various concentration of isolated compounds in 60,80 and 100 mg/ml. Filter paper disc loaded with 10µl of amoxicillin was used as positive control. Negative control was prepared by using ethanol as solvent. These plates were incubated at 37˚C for 24 hours and the zone of inhibition was noted in millimetre **[18]**.

3. RESULT AND DISCUSSION

3.1. QUALITATIVE ANALYSIS OF FLOWER AND STEM OF *COSTUS IGNEUS*

The flower and stem extract of *Costus igneus* showed the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, phlobatannins, proteins, coumarins, emodin, anthroquinone, anthocyanin, carbohydrate, leucoantiocyanin, cardiac glycosides, xanthoprotein, phenols. The qualitative analysis of the phytochemical constituents which have various pharmacological activities (**Table 3.1.a**).

Dr C. Muthukumar *et al.,* 2019 **[18]** estimated that the leaf extract of *Costus igneus* contains alkaloids, carbohydrates, reducing sugar, saponin, protein, phenolic compounds, tannin and glycosides. Rajani Chowdary *et al*., 2020 **[19]** showed the presence of phytochemicals in leaf of *Costus igneus* which include flavonoid, alkaloids, terpenoids, tannins, steroids, quinones, polyphenol, phenol, saponins, glycosides and coumarins. The phytochemicals screening of *Costus pictus* leaves extract showed that presence ofalkaloids, glycosides, polysaccharides, tannins, saponins and phenolic flavonoid, terpenoids carotenoids and steroids analysed the qualitative phytochemicals (N. Jothivel, *et al.,* 2007) **[20]**.

3.2. QUANTITATIVE ANALYSIS OF *COSTUS IGNEUS* FLOWER AND STEM EXTRACT (Table 3.1.a)

The phytochemical analysis of *Costus igneus* flower extract contains the highest

concentration of flavonoid (0.006mg /g), tannin (0.013mg /g), saponin (0.021mg /g), alkaloids (0.093mg /g), phenol (0.013 mg /g), terpenoids (0.008mg /g) and stem extract of *Costus igneus* showed the presence of various phytochemicals at the concentration of flavonoid (18.139g), tannins (19.920g), saponin (19.576g), alkaloids (20.369g), phenol (20.795g) and terpenoids (20.916g). (Dr.C. Muthukumar *et al*., 2019) evaluated that the quantitative determination of secondary metabolites from *Costus igneus* contains alkaloids 14.5±0.1124mg/g in the extract, saponin content was 61.1±0.0823mg/g, flavonoid content was 58.3±0.2837mg/g. **(Rajani Chowdary *et al*., 2020)** stated that the phytochemicals present in leaf extract of *Costus igneus* such us protein content (4.24± 0.4), reducing sugar (0.07µg/ml) **Table 3.2.a**.

3.3. VISUAL COLOUR CHANGE AND UV- VISIBLE SPECTROSCOPY

In UV visible spectroscopy analysis, the colorless solution was changed into reddish brown color by adding the ethanol extract of *Costus igneus* flower and stem sample into the AgNPs. This showed the plasma resonance band which was observed at the range of 436nm for flower and 430nm for stem by UV spectra **Table 3.3.a, b**.

3.4. FUNCTIONAL GROUP DETERMINATION USING FT-IR SPECTROSCOPY

FT-IR spectroscopy analysis provides the information about the functional groups. The FT-IR spectra of synthesized silver nanoparticles showed the absorption peaks for flower of *Costus igneus* at 3450.45cm^{-1} corresponds to N-H stretching 2923.30cm^{-1}, 2125.96cm-1 corresponds to C=C stretching vibration, 1416.19cm-1,1384.24cm^{-1} corresponds to C-O stretching, 1113.57cm^{-1}, corresponds to C-N stretching vibration and 617.51cm^{-1} corresponds to C-Br stretching vibration and the absorption peaks for stem of *Costus igneus* at 3450.45 (O-H stretching and H-bonded), 2852.47 (C-N in aliphatic amine), 1639.13 (C-D stretching in aliphatic ether), 1113.57 (-C=C- in alkenes), 617.51 (medium C=C stretching conjugated alkenes) **(Figure 3.4.a, b)**.

3.5. SEM (scanning electron microscope)

The size and shape of the synthesized AgNPs was analyzed by using SEM technique. SEM image of silver nanoparticles synthesized from flower of *Costus igneus* the size of the particle range was 94.3-116.8nm in diameter and size was about 200nm. The **Figure 3.5 a**: a showed the size and shapesof synthesized silver nanoparticles from the flower and stem extract of *Costus igneus* was found to be 178.5 and 218.6 nm andthe shapes are spherical and cubic. The average size of nanoparticles is 200nm. Whereas the shapes are spherical and cubic was shown in **Figure 3.5 b**.

3.6.EDX analysis

Energy dispersive x-ray (EDX) was used for elemental analysis where the x-ray counts was observed at y axis and Kev (energy) was observed at x axis. The biomolecules attached to the AgNPs shows the chloride peaks. The Ag weight of the flower sample is about 78.24% and the silver weight of the stem sample is about 65.8%. This shows the reduction of AgNPs **Figure 3.6.a** shows SEM image for synthesized silver nanoparticles for *Costus igneus* (stem).

3.7. X-RAY DIFFRACTION (XRD)

The XRD pattern of AgNPs was used to confirmed the crystalline nature of AgNPs. The diffraction peaks are obtained at 20 values 38.36, 46.69, 68.32, 78.44 corresponds to (111), (200), (220), (311). This reflection planes of face centered cubic structure of silver according to (JCPDS, file No 0.4-0.783). The flower extract of *Costus igneus* containing the phytochemical was shows this unassigned peak. The X- ray diffractogram of synthesized silver nanoparticles for stem extract showed that peaks are obtained at 20ranges. 38.3467, 45.5823, 65.1648, 78.4517, corresponds to diffraction facets are (111), (200), (220), (311) respectively. This pattern reflection of face centred cubic structure of silver according to (JCPDS, file no 04-0783) **Figure 3.7.a, b**.

3.8. ANTIOXIDANT ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES USING FLOWER AND STEM OF *COSTUS IGNEUS* BY DPPH ASSAY

The synthesized silver nanoparticles of *Costus igneus* flower extract and stem extract was compared with ascorbic acid. The antioxidant activity of *Costus igneus* flower extract was increases with increase in concentration. The synthesized silver nanoparticles of *Costus igneus* flower extract contains high concentration of antioxidant activities when compared with *Costus igneus* stem extract. This showed the antioxidant activity of synthesized silver nanoparticles using *Costus igneus* flower extract was found to be 81.3% and the antioxidant activity of synthesized silver nanoparticles using *costus igneus* stem extract was found to be 79.6% at concentration 100µg/ml while ascorbic acid gave 91.52% at the same concentration **(Table 3.8 a; Figure 3.8.a)**.

(Bhat.V *et al*., 2010) **[26]** suggested that leaf of *Costus mexicanus* have the antioxidant activity of 89.5% and 90.0% when compared with butylated hydroxy toluene 85%µg/ml. (NimmyChacko *et al.,* 2018) **[21]** reported that the *Costus igneus* plant extract produced 71.85% DPPH scavenging activity compared to ascorbic acid which produced 84.47%. (Naik A *et al*., 2017) **[22]** revealed that the leaf extract of

Costus pictus having the DPPH radical scavenging by CPAQ and CPME at 1mg/ml was 81.25% and 89.25% with EC50 values were 0,468 and 0.360 mg/ml, respectively while ascorbic acid has 99.87% activity at 1concentration. (Sun.J.*et al.,*2002) **[23]** revealed that present of antioxidant activity of *Costus igneus* leaves extract using four methods DPPH assay reducing power assay superoxide radical scavenging assay and folinciocalteu assay. The proved that extract antioxidant activity reducing power assay showed 75.43%. Ascorbic acid showed 91.94%. DPPH assay plant extract 71.85%. DPPH scavenging activity superoxide scavenging activity produced 68.19%radical scavenging activity produced 68.19% radical scavenging activity 79.78% *Costus igneus leaf* extract against antioxidant activity.

3.9. INVITRO ALPHA AMYLASE ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLE VS STANDARD ACARBOSE

The synthesized silver nanoparticles of *Costus igneus* flower extract and stem extract was compared with standard drug acarbose. The alpha amylase inhibitory action of synthesized silver nanoparticles from the *Costus igneus* flower extract was increases with increase in concentration. The synthesized silver nanoparticles of *Costus igneus* flower extract contain high concentration of alpha amylase activity when compared with *Costus igneus* stem extract. This showed the alpha amylase inhibitory action of synthesized silver nanoparticles using *Costus igneus* flower extract was found to be56.0% to 84.9% at concentration of 20-100µg/mland thealpha amylase activity of synthesized silver nanoparticles using *costus igneus* stem extract was found to be 50.7 to 82.5% at concentration 20-100µg/ml while acarbose gave 69.1 to 85.3% at the same concentration **(Table 3.9 a; Figure 3.9.a)**.

3.10. INVITRO ALPHA GLUCOSIDASE ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES VS STANDARD ACARBOSE

The synthesized silver nanoparticles of *Costus igneus* flower extract and stem extract was compared with standard drug acarbose. The alpha glucosidase activity of synthesized silver nanoparticles from the *Costus igneus* flower extract was increases with increase in concentration. The synthesized silver nanoparticles of *Costus igneus* flower extract contain high concentration of alpha glucosidase activity when compared with *Costus igneus* stem extract. The synthesized nanoparticles are used to study for alpha glucosidase under in vitro condition reported maximum inhibition at 20-100µg/ml the percentage of inhibition varied from (45.3 to 58.3µg/ml) for flower and for stem (65.7 to 79.6%) high concentration to low concentration. For the

comparison study acarbose is used as a standard drug for α-glucosidase, where the acarbose concentration (20-100µg/ml) showed that the activity from (65.7-79.6µg/ml) **(Table 3.10 a; Figure 3.10.a)**.

(Handunge Kumudu Irani Peraera *et al.*, 2016) [29] suggested that the alpha glucosidase activity of the COS leaves with a IC_{50} of 67.5µg/ml which was significantly lower than acarbose and alpha amylase activity of the COS leaves have the alpha amylase inhibitory concentration was 5.88mg/ml which was higher. the antidiabetic activity of *Costus pictus* flower extract using α-amylase and α-glucosidase activity tested. α-amylase showed that (20-100µg/ml) of various concentration 40.879 to 25.772 %at silver nanoparticles. (7o.00 to 83.47 %) at acarbose concentration and α-glucosidase activity are various concentration of (20-100µg/ml) at silver nanoparticles (56.26 to 58.72%) and (66.32 to 86.61%) at acarbose concentration.

3.11. ANTIPERIDONTAL ACTIVITY OF SYNTHESIZED AgNPs FROM FLOWER AND STEM OF *COSTUS IGNEUS*

The substandard quality of oral cavity health leads to chronic diseases in humans. The species which are prone to oral health includes *Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus* and *Staphylococcus aureus*. The maximum inhibition was absorbed against *Streptococcus salivarius, Staphylococcus aureus* is 7mm in diameter and *streptococcus mutans, streptococcus sobrinus* are 6mm in diameter. The antiperiodontal activity of synthesized AgNPs against these bacteria was investigated and results were tabulated in the (**Table 3.11 a)**. The zone of inhibition of these bacterial strains is shown in **(Figure 3.11 a, b)**.

(Yogita sardessai *et al*., 2014) **[30]** estimated that the antimicrobial activity of methanolic extract of the rhizomes of *Costus igneus* such as *staphylococcus aureus* have the percentage inhibition of growth at concentration of 29.41%, *bacillus subtilis* 57.14%, *Pseudomonas aeruginosa* 48.27%.

Table 3.1.a: Qualitative analysis of flower and stem of *Costus igneus*

S. No.	PHYTOCHEMICAL CONSTITUENTS	*Costus igneus* (flower) (mg/g)	*Costus igneus (stem) (mg/g)*
1.	Flavonoid	0.006	0.002
2.	Tannin	0.013	0.007
3.	Saponin	0.021	0.009
4.	Phenol	0.013	0.007
5.	Alkaloids	0.093	0.003
6.	Terpenoids	0.008	0.007

+ - Trace ++ - Moderate +++ - Strong A – Absence

Table 3.2.a: Quantitative Analysis of Ethanolic Extract Of Flower And Stem of *Costus igneus*

Test no	Test for	Observation	Flower	Stem
1	Terpenoids	Reddish brown	+++	+
2	Flavonoid	Yellow color	+++	+++
3	Saponin	Formation of emulsion	+++	+++
4	Tannin	Green precipitate	+++	+++
5	Alkaloids	Yellow color	+++	+++
6	Steroids	Reddish brown ring	+++	+++
7	Glycosides	Violet, blue, green	+++	+++
8	Phlobatannins	Redprecipitate	+++	+
9	Protein	White precipitate	+++	+++
10	Coumarin	Yellow color	+++	+++
11	Emodin	Red color	++	+++
12	Anthroquinone	Pink, Violet or red	++	+++
14	Anthocyanin	Pinkish red to blueish violet	+++	+++
14	Carbohydrate	Reddish violet	+++	+++
15	Leucoantiocyanin	Red color	+++	+++
16	Cardiac glycosides	Brown ring / Violet	+++	+++
17	Xanthoprotein	Blue /black	+++	+++
18	Phenol	Reddish / orange	+++	+++

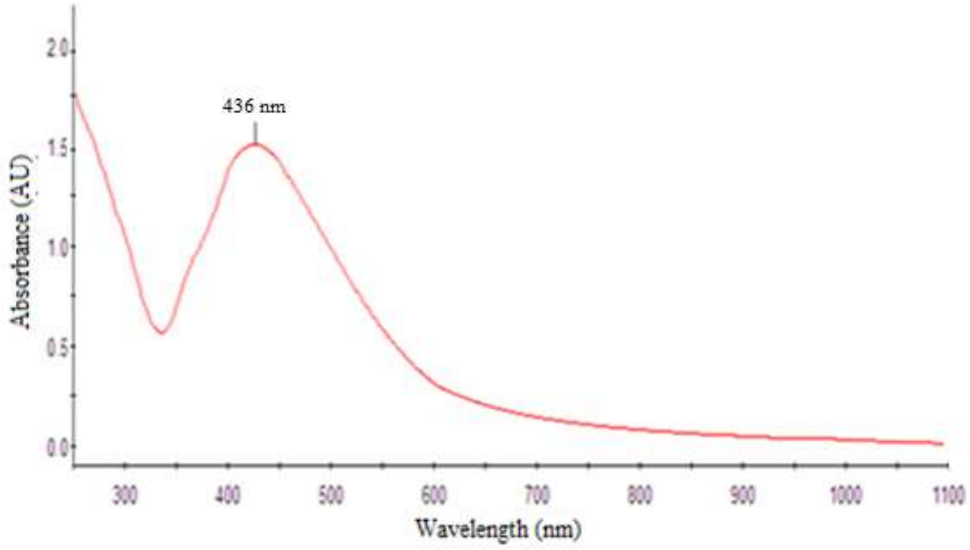

Figure 3.3.a: Visual observation of synthesized silver nanoparticles for flower of *Costus igneus*

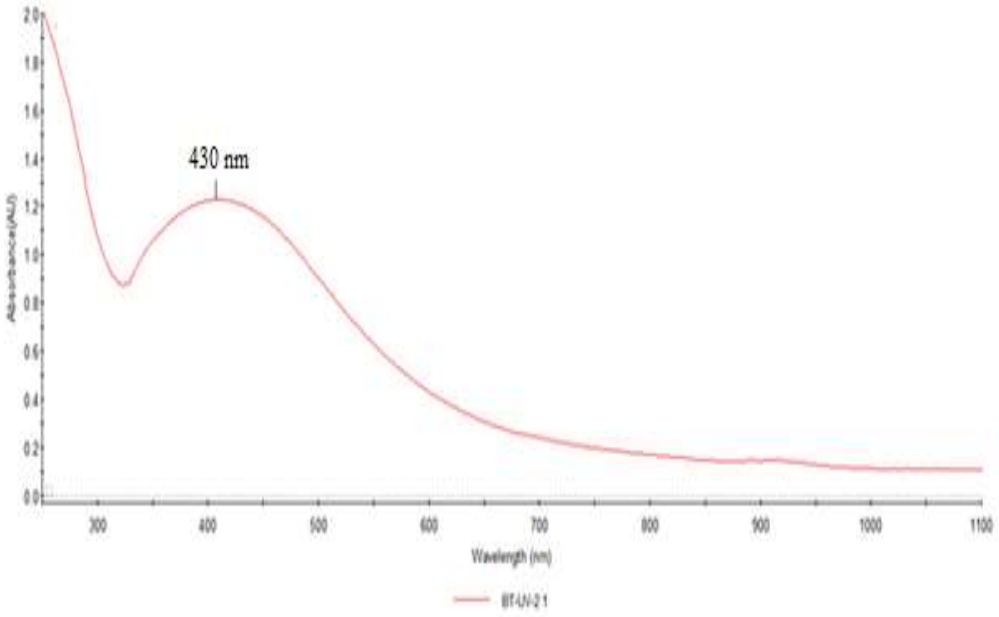

Figure 3.3.b): Visual observation of synthesized silver nanoparticles for stem of *Costus igneus*

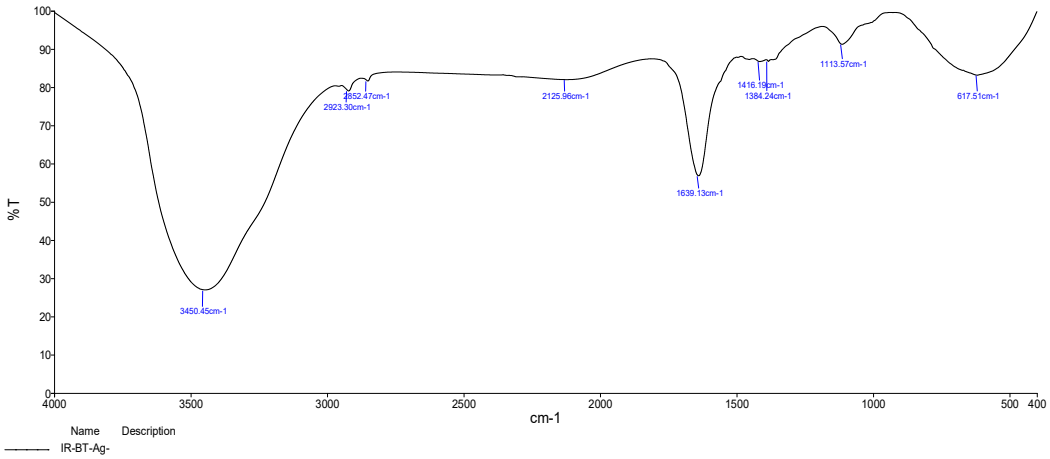

Figure 3.4.a: FT-IR spectra of synthesized silver nanoparticles for flower of *Costus igneus*

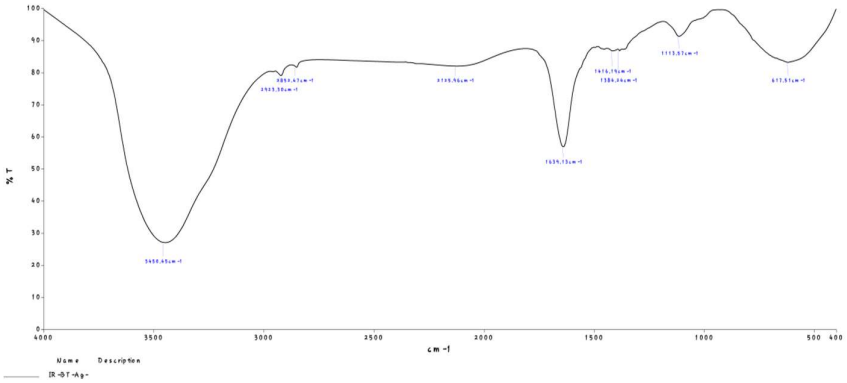

Figure 3.4.b: FT-IR spectra of synthesized silver nanoparticles for stem of *Costus igneus*

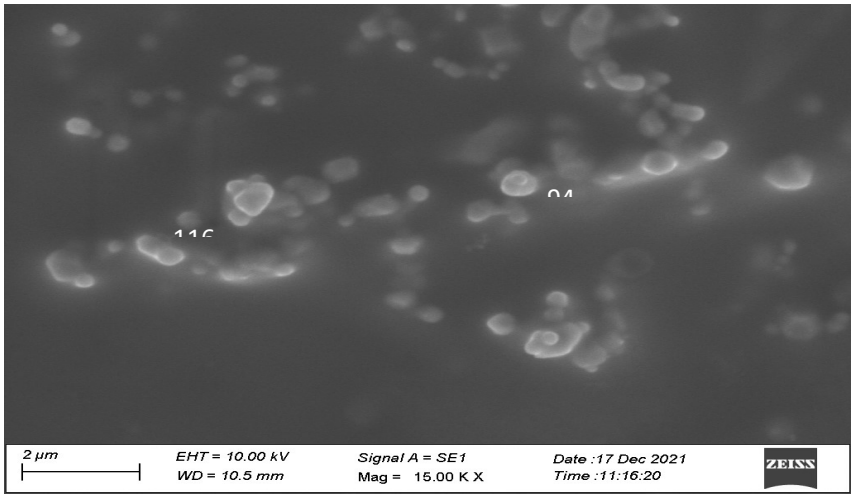

Figure 3.5.a: SEM image for synthesized silver nanoparticles for *Costus igneus* (flower)

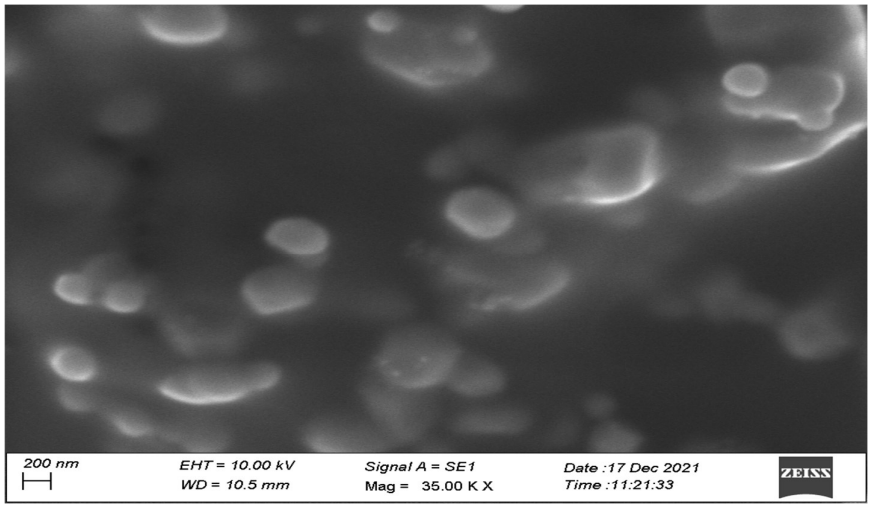

Figure 3.5.b: SEM image for synthesized silver nanoparticles for *Costus igneus* (stem)

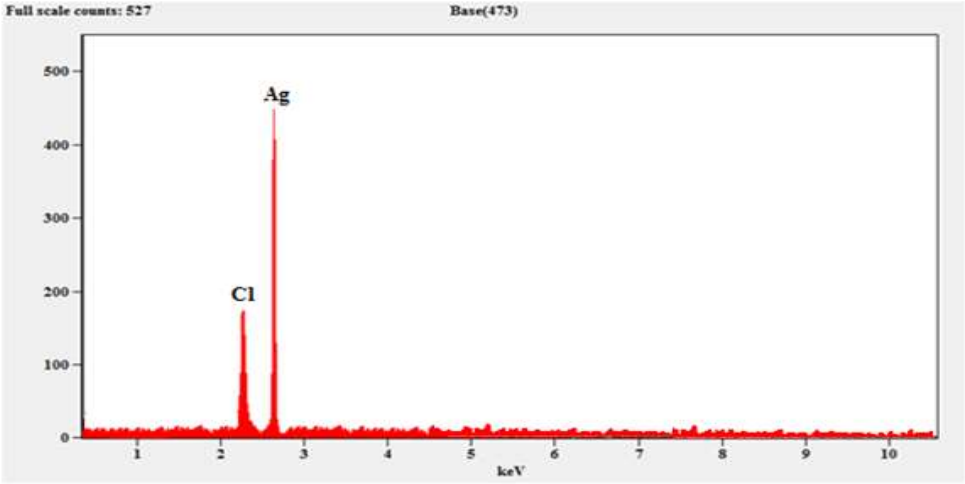

Figure 3.6.a: EDX spectrum of synthesized silver nanoparticles for *costus igneus* (flower)

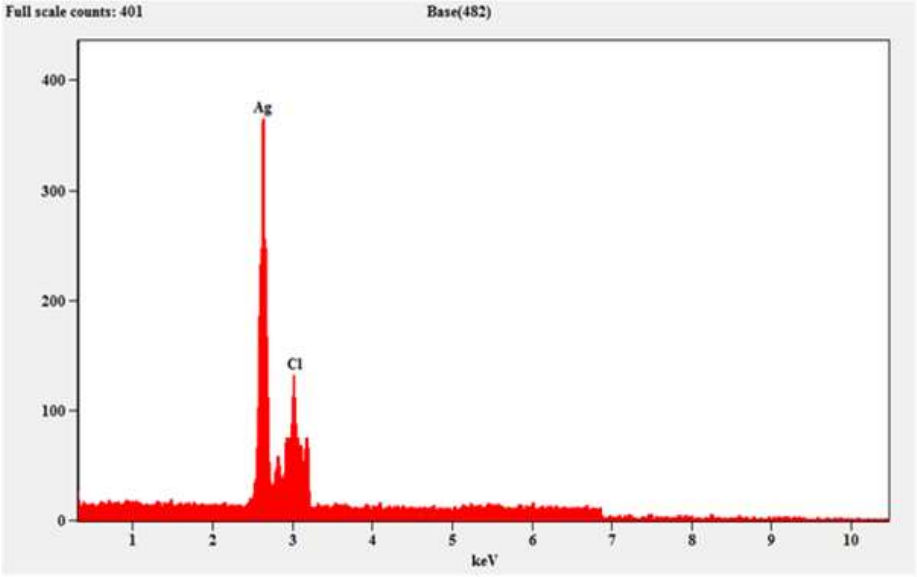

Figure 3.6.b: EDX spectrum of synthesized silver nanoparticles for *costus igneus* (stem)

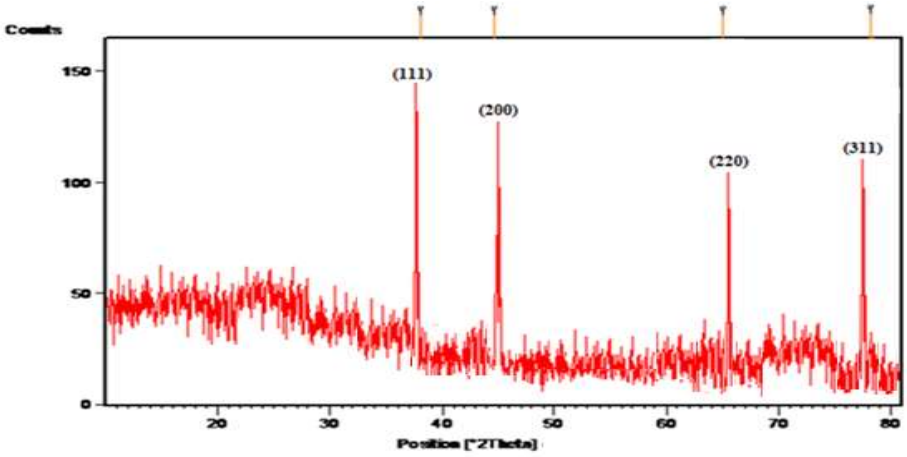

Figure 3.7.a: XRD spectrum of synthesized silver nanoparticles for *costus igneus* flower

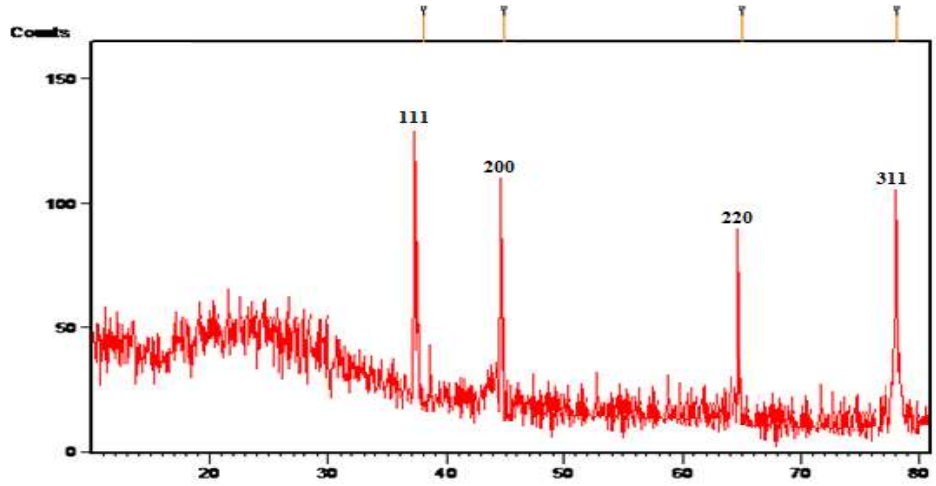

Figure 3.7.b: XRD spectrum of synthesized silver nanoparticles for *costus igneus* stems

Table 3.8.a: Antioxidant activity of synthesized silver nanoparticles using flower and stem of *Costus igneus* by DPPH activity

S. No.	CONCENTRATIONS	SCAVENGING EFFECT (%)		
		SILVER NANOPARTICLES (flower)	SILVER NANOPARTICLES (STEM)	ASCORBIC ACID
1	20(µg/ml)	66.9	55.1	82.2
2	40(µg/ml)	72.0	63.5	85.5
3	60(µg/ml)	75.7	74.5	87.2
4	80(µg/ml)	77.5	77.9	89.8
5	100(µg/ml)	81.3	79.6	91.5

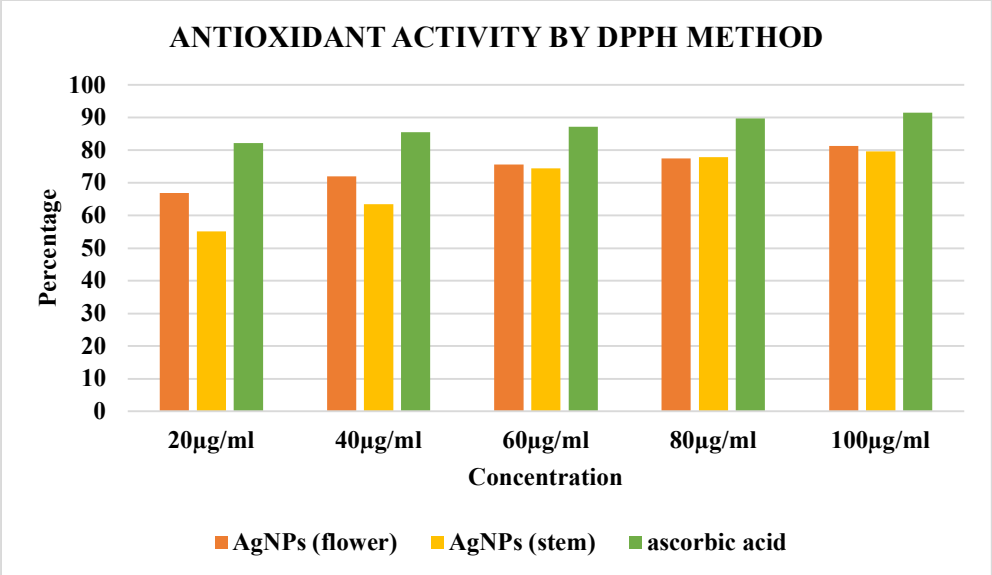

Figure 3.8.a: Antioxidant activity of synthesized silver nanoparticles using flower and stem of *Costus igneus* by DPPH method.

Table 3.9.a: In vitro alpha amylase activity of synthesized silver nanoparticles vs standard acarbose.

S. No,	CONCENTRATIONS	ALPHA AMALYSE%		
		AgNPs (FLOWER)	AgNPs (STEM)	ACARBOSE
1	20(µg/ml)	56.0	50.7	69.1
2	40(µg/ml)	74.6	59.3	78.0
3	60(µg/ml)	78.5	67.2	80.4
4	80(µg/ml)	80.9	79.1	83.7
5	100(µg/ml)	84.9	82.5	86.3

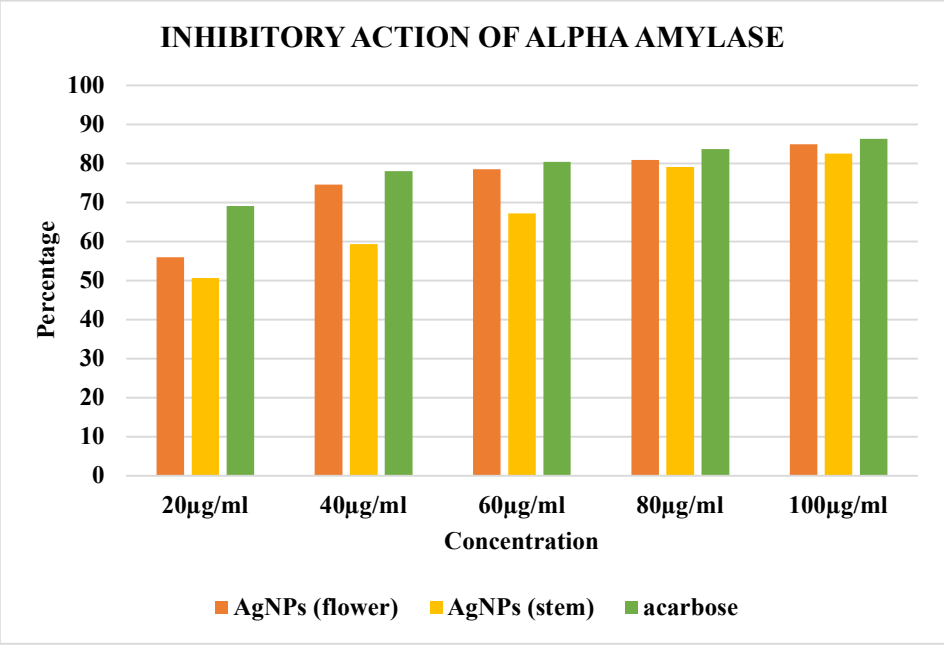

Figure 3.9.a: In vitro alpha amylase activity of synthesized silver nanoparticles vs standard acarbose

Table 3.10.a: In vitro alpha glucosidase activity of synthesized silver nanoparticles vs standard acarbose

S. No.	CONCENTRATIONS	ALPHA GLUCOSIDASE (%)		
		AgNPs (Flower)	AgNPs (stem)	ACARBOSE
1	20 (µg/ml)	45.3	39.8	65.7
2	40 (µg/ml)	48.1	41.6	68.5
3	60 (µg/ml)	51.8	46.2	73.1
4	80 (µg/ml)	55.5	50.0	75.9
5	100 (µg/ml)	58.3	53.7	79.6

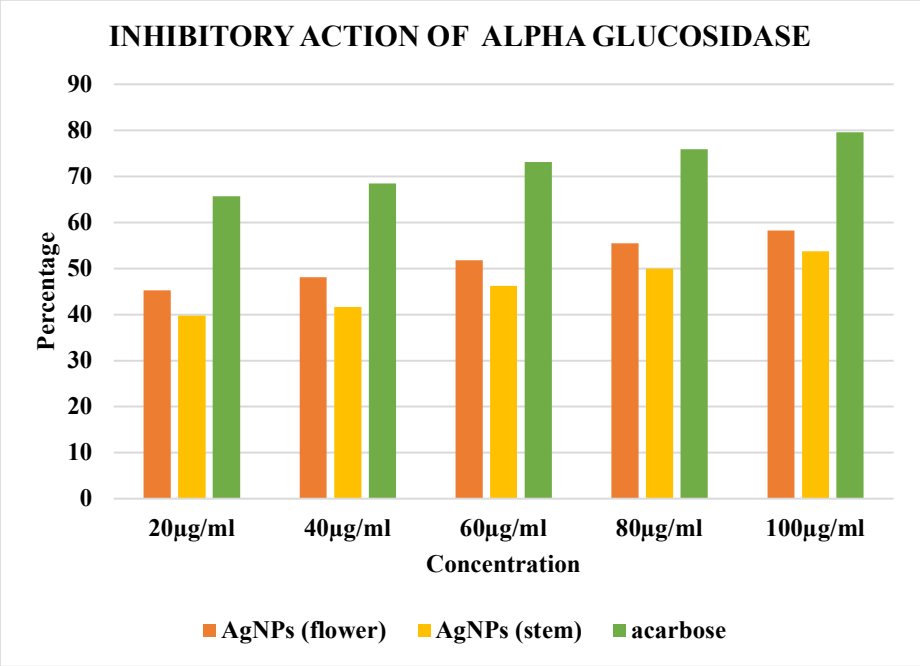

Figure 3.10.a) In vitro alpha glucosidase activity of synthesized silver nanoparticles vs standard acarbose

Table 3.11.a: Antiperiodontal activity of Synthesized AgNPs from flower and stem of *Costus igneus*

Samples	Concentration (µg/ml)	Organisms/ Zone of Inhibition (mm)							
		Isolated Organisms							
		Streptococcus mutans		*Streptococcus salivarius*		*Streptococcus sobrinus*		*Staphylococcus Aureus*	
		B1(F)	B1(S)	B2(F)	B2(S)	B3(F)	B3(S)	B4(F)	B4(S)
Standard (Amoxicillin)	S	8	7	10	11	9	10	11	8
Synthesized AgNPs	60	3	2	3	3	2	2	2	2
	80	4	4	5	5	4	3	5	4
	100	6	5	7	7	6	4	7	6

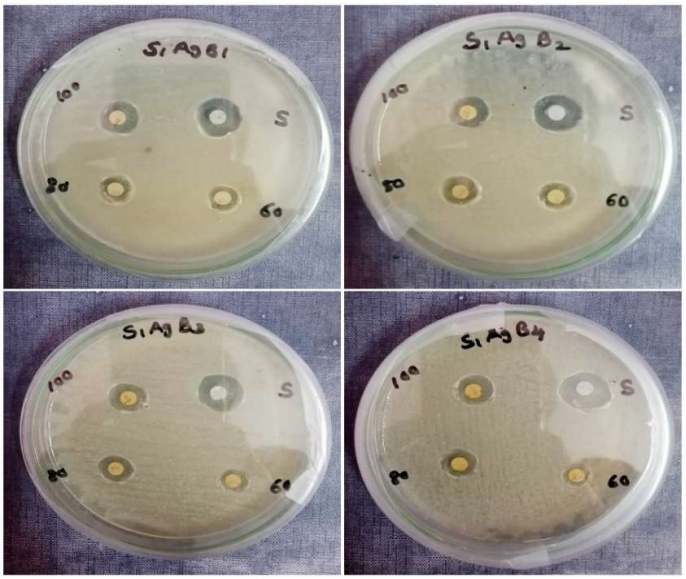

Figure 3.11.a) Zone of inhibition observed in antiperiodontal activity

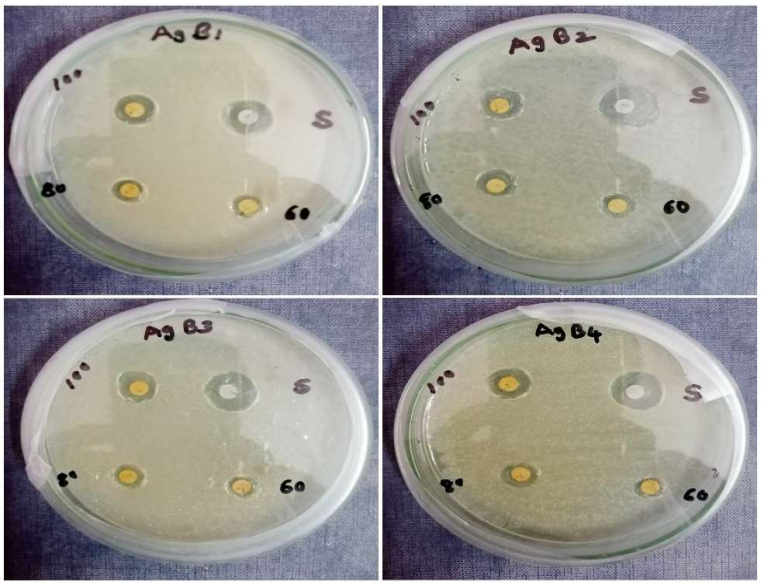

Figure 3.11.b

3.4. CONCLUSION

The aim of antidiabetic and antiperiodontal activity of synthesised silver nanoparticles from *Costus igneus* flower and stem extract. Biological method is using because safe, inexpensive, eco-friendly. AgNPs synthesized using stem and flower of *Costus igneus* characterized UV-Visible spectrum showed an absorption peak at 430nm. The FTIR analyzed the silver nanoparticles showed that the biological material and functional group present in the flower and stem AgNPs of *Costus igneus*. The synthesized nanoparticles stem and flower of *Costus igneus* revealed that antioxidant anti-diabetic and anti-periodontal activities are more effectively. The antioxidant activity AgNPs synthesized using *Costus igneus* flower and stem extract showed high compared to ascorbic acid. The antioxidant activity of synthesized AgNPs showed 79.66% at concentration 100µg/ml while ascorbic acid 91.52% at the same concentration. The inhibitory action ofα-amylase by synthesized AgNPs from *Costus igneus* stem and flower extract was showed 27.22% at concentration 100µg/ml and acarbose 85.36% at same concentration. The α-glucosidase activity of AgNPs synthesized using *Costus igneus* stem and flower extract was showed high compared to acarbose. The alpha glucosidase activity of synthesized AgNPs showed 58.33% at concentration 100µg/ml and acarbose 87.62% at same concentration. The anti-periodontal activity of synthesized silver nanoparticles of *Costus igneus* stem and flower was found to be high (B2-7).

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