The In-vitro Antidiabetic and Antioxidant Activity of Silver Nanoparticles Synthesized From *Chrysanthemum indicum* Flower Extracts

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Abstract

The present study aimed to assess the antidiabetic and antioxidant potential of green synthesized Silver nanoparticles from different extracts of *Chrysanthemum indicum.* The Ethanolic, Petroleum ether, and Hydro-alcoholic flower extracts were used to synthesize Silver nanoparticles. The DPPH free radical scavenging assay was performed in Silver nanoparticles synthesized from three plant extracts, among the nanoparticles synthesized, the AgNPs synthesized from hydro-alcoholic extract shows the highest scavenging of DPPH radical comparable to the standard ascorbic acid. The IC50 value of AgNPs synthesized from hydro-alcoholic extract was 62.03 µg/ml and the standard Ascorbic acid was 52.0 µg/ml. The

in-vitro antidiabetic activity of AgNPs synthesized from Ethanolic, Petroleum Ether, and Hydro-alcoholic flower extracts was studied. The AgNPs synthesized from hydroalcoholic flower extract shows good antidiabetic activity comparable to other extracts. The Alpha Glucosidase inhibitory assay revealed the IC50 value of AgNps synthesized from Ethanolic extract was 72.35 µg/ml, Petroleum Ether was 79.23 µg/ml and Hydro alcoholic was 60.05 µg/ml and compared with standard Acarbose 50.25 µg/ml. The AgNPs synthesized from Hydro alcoholic flower extract shows good antidiabetic activity. In the Alpha -amylase inhibitory assay, the IC50 value of AgNps synthesized from Ehanolic extract was 80.32 µg/ml, Petroleum Ether extract was 93.05 µg/ml and Hydroalcoholic extract was 65.23 µg/ml and the standard Acarbose was 50.25 µg/ml. The AgNps synthesized from Hydroalcoholic flower extract expressed its best result 47.16±0.96 at the 1mg/ml concentration. The AgNps synthesized from Hydroalcoholic flower extract showed higher inhibitory potential against diabetes.

Keywords: Silver Nanoparticles, *Chrysanthemum indicum*, free radical, antidiabetic, antioxidant.

Introduction

Diabetes mellitus is the most common endocrine metabolic disorder and causes significant morbidity and mortality.[1] Hyperglycemia causes retinopathy, neuropathy, nephropathy, and other macrovascular complications.[2] Diabetes mellitus is growing rapidly in developing countries. About 693 million people in this world will be affected by diabetes in 2045.[3] The non-insulin dependent diabetes called type 2 diabetes is the most common and in which the body does not produce sufficient insulin or the insulin is not properly utilized.[4] Many anti-diabetic drugs and gliptins are used to treat diabetes but they cause various side effects such as nasopharyngitis, headache, nausea, hypersensitivity, and skin reactions.[5-7] These complications lead to finding suitable antidiabetic drugs to treat diabetic mellitus. The flower extract of *Chrysanthemum indicum* possesses various phytochemicals such as coumarins, Flavonoids, Tannins, Anthraquinones, Steroids, Cardia Glycosides, Terpenoids, Alkaloids, Saponins, and Phenol. Due to the presence of phytochemicals, the flower extract of *Chrysanthemum indicum* showed antidiabetic activity. This current study was focused on preparing AgNps from different flower extracts of Chrysanthemum indicum and evaluating its antidiabetic and ant-oxidant activities by in-vitro model.

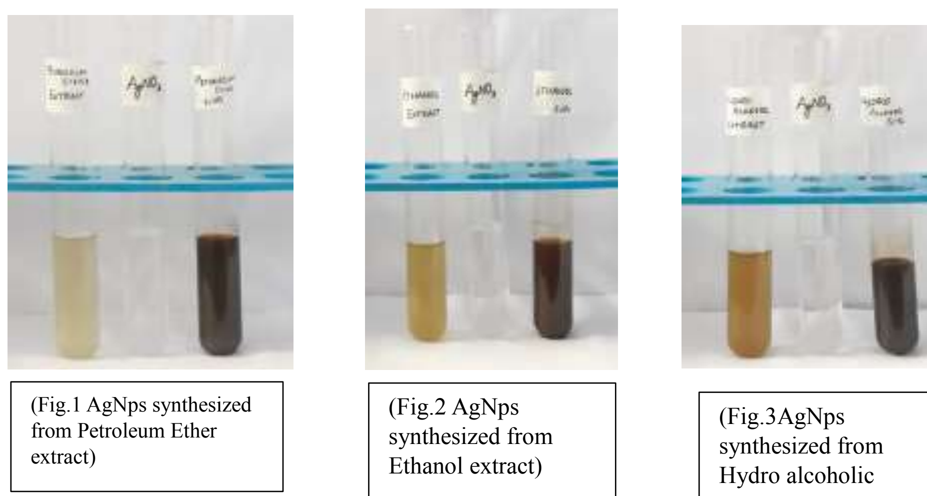
Materials and Methods

The flower samples were washed with double distilled water and dried in the shade and ground into powder in a medicinal flavor mill. About 50g of powder was packed by making a thimble with Whatman No.1 filter paper. The thimble was placed in a 200ml extractor of Soxhlet apparatus and extracted with different solvents such as petroleum Ether, Ethanol, and Hydro alcoholic (50%) for at least 16 reflux for 12 hours. The resultant extracts were concentrated using a rotary evaporator (Buchi. Switzerland) at vacuum conditions. The extracts were stored at 4◌̊ C for further experiments.

Synthesis of Silver nanoparticles

The different solvent extracts were used for the bioreduction process. To synthesize nanoparticles 1 ml of plant extract solubilized in Ethanol, Petroleum Ether, and

Hydroalcoholic solvents (100 mg/mL) was carefully added to 10 mL of 1 mM aqueous $AgNO_3$ solution in a 25 mL test tube. The flask containing extracts was incubated in a shaker at 150 rpm in dark conditions. The bioreduction of nanoparticles by $AgNO_3$ was monitored at 24 h by UV-vis spectroscopy. The reduced silver solution was centrifuged at 10,000 rpm for 15 minutes and the pellet was washed three times with 20 mL of deionized water. Silver nanoparticles were synthesized using all three different solvent extracts and they were turned brown in color due to the reduction of the elements by co-precipitation and capping action by the plant extracts.



(Fig.1 AgNps synthesized from Petroleum Ether extract)

(Fig.2 AgNps synthesized from Ethanol extract)

(Fig.3AgNps synthesized from Hydro alcoholic

Figure 1: Synthesis of silver nanoparticles using Petroleum ether, Ethanol and Hydroalcoholic flower extract of *Chrysanthemum indicum*

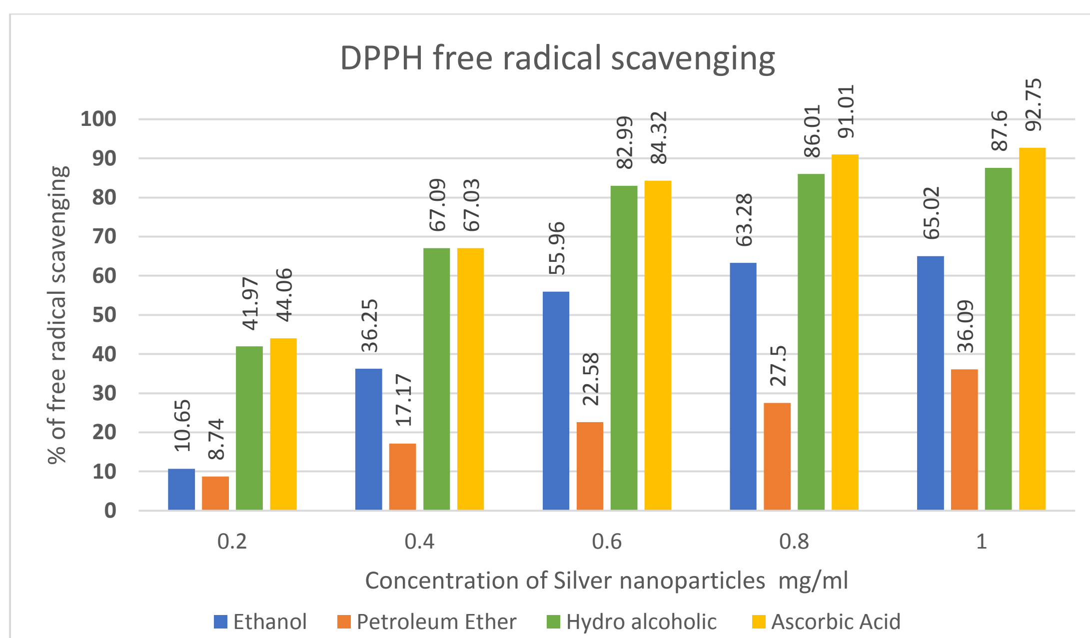
Invitro antioxidant activity

The DPPH assay was carried out on Silver nanoparticles synthesized from Ethanol, Petroleum Ether, and Hydro alcoholic flower extracts of *Chrysanthemum indicum.* About 2ml of DPPH solution was added to 0.2mL of various concentrations of the AgNps of Ethanolic, Petroleum Ether, and Hydro alcoholic samples. The mixture was vortexed and kept at room temperature for 30 minutes in the dark to allow scavenging of DPPH radical . The scavenging percentages of DPPH were calculated by using the following formula.

$$\text{Free radical scavenging (\%)} = \frac{[A_c - A_s]}{A_c} \times 100$$

Here the A_c is the absorbance of the DPPH control and $A_s$is the Absorbance of the DPPH. [12-13]The result showed that scavenging potential increased when the concentration of the AgNps was increased.

S.No	Concentration mg/ml	% of inhibition by Ascorbic acid (Standard)	% of inhibition by AgNps synthesized from Ethanolic flower extract	% of inhibition by AgNps synthesized from Petroleum Ether flower extract	% of inhibition by AgNps synthesized from Hydroalcoholic flower extract
1	0.2	44.06±0.2	10.65±0.98	8.74 ±0.32	41.97 ±0.23
2	0.4	67.03±0.21	36.25±0.12	17.17 ±1.08	67.09 ±0.12
3	0.6	84.32±0.71	55.96±0.56	22.58 ±0.78	82.99 ±0.56
4	0.8	91.01±0.61	63.28±0.87	27.50 ±0.54	86.01 ±0.48
5	1	92.75±0.30	65.02±0.91	36.09 ±0.36	87.60 ±0.96
IC 50 value		52.03 µg/ml	75.68 µg/ml	90.01 µg/ml	62.08 µg/ml



The AgNps synthesized from Hydro alcoholic flower extract of *Chrysanthemum indicum* showed a remarkable result of 67.09 ±0.12 mg/mL in 0.4 mg/mL concentration similar to the Standard Ascorbic acid. The IC 50 value was 62.08 ug /ml. The AgNps synthesized from Hydro alcoholic flowers showed better results than Ethanol and Petroleum Ether.

Antidiabetic activity

Alpha Amylase inhibitory assay

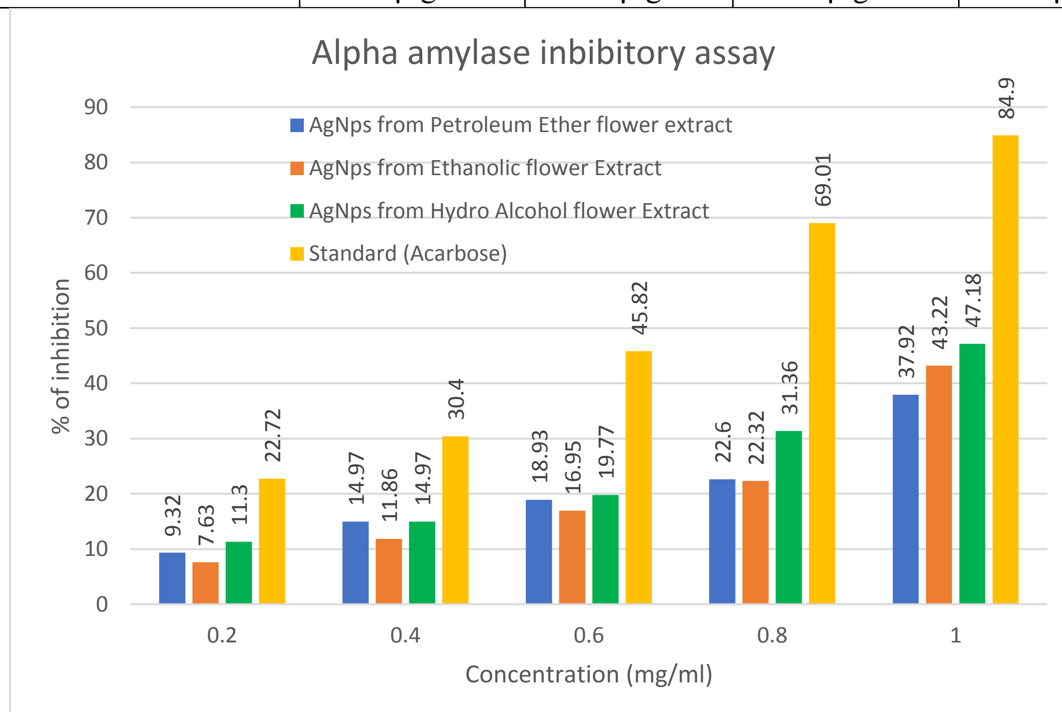
The enzyme alpha-amylase breaks down the polysaccharides and releases the glucose into the blood stream. It leads to hyperglycemia. The AgNps exhibits the inhibitory effect on the enzyme in a dose-dependent manner. The assay mixture containing 200ul of 0.02M Sodium phosphate buffer, 20 ul of the enzyme, and the AgNps concentration range 20-200

ug/ml were incubated at room temperature for 10 minutes followed by the addition of 200ul. lf starch in all test tubes. The reaction was terminated with the addition of 400µl DNS reagent and placed in a boiling water bath for 5 minutes cooled and diluted with 15ml of distilled water and absorbance was measured at 540nm. The control samples were prepared without any plant extracts. The % of inhibition was calculated according to the formula [14]

$$\text{The inhibition \%} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Table: Alpha-amylase inhibitory potential of different silver nanoparticles

S.No	Concentration mg/ml	% of inhibition by Ascorbic acid (Standard)	% of inhibition by AgNps synthesized from Ethanolic flower extract	% of inhibition by AgNps synthesized from Petroleum Ether flower extract	% of inhibition by AgNps synthesized from Hydroalcoholic flower extract
1	0.2	22.72±0.23	7.63±0.21	1.98 ±0.21	11.30±0.20
2	0.4	30.40±0.33	11.86±0.36	3.67 ±0.39	14.97 ±0.36
3	0.6	45.82±0.57	16.95±0.45	9.04±0.46	19.77 ±0.48
4	0.8	69.01±0.70	22.32±0.58	10.45 ±0.36	31.36 ±0.58
5	1	84.90±0.39	43.22±0.79	14.12 ±0.75	47.18 ±0.96
IC 50 value		50.32 µ g/ml	80.32 µ g/ml	93.05 µ g/ml	65.23 µ g/ml

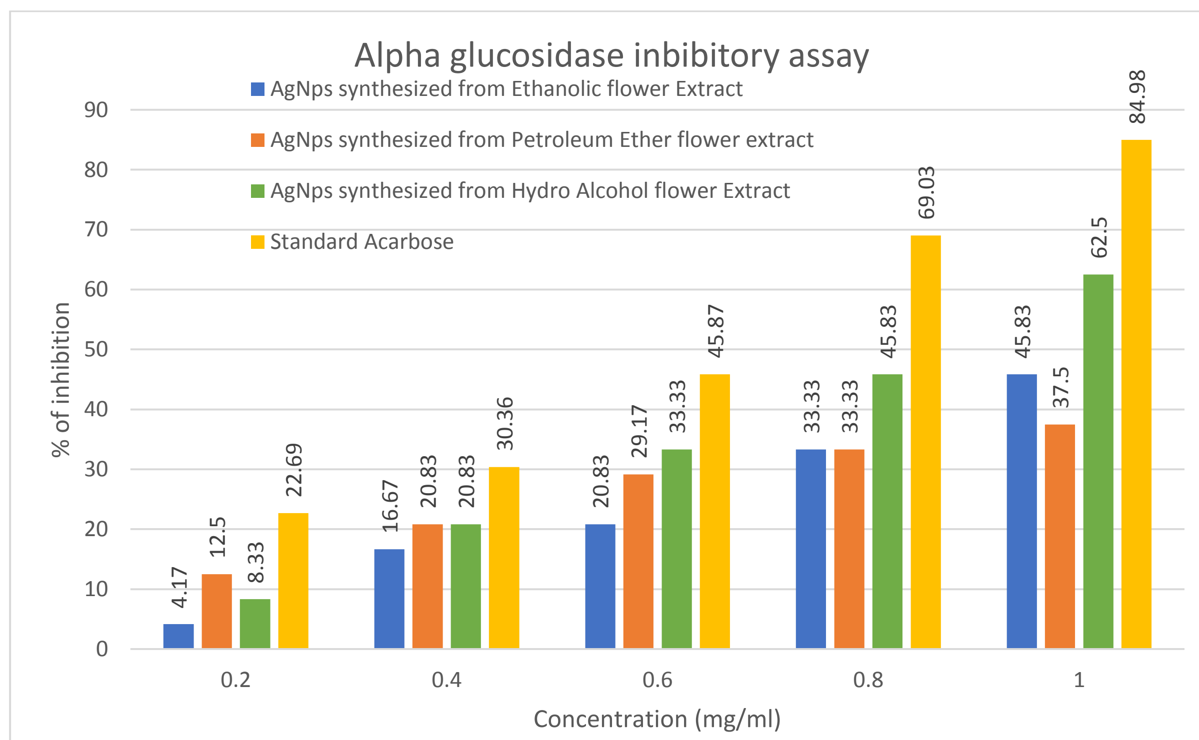


Alpha Glucosidase inhibitory assay

The enzyme alpha-glucosidase catalyzes the disaccharides into monosaccharides which leads to postprandial hyperglycemia. The alpha-glucosidase inhibitory assay was conducted with the AgNps synthesized from Ethanolic, Petroleum Ether, Hydro alcoholic, and Acarbose which was the standard. Yeast alpha-glucosidase (0.7 U) dissolved in 100 mM phosphate buffer (PH 7.0) containing 2g/l bovine serum albumin, and0.2g/l NaN3, and 5mM nitrophenyl-a-D-glucopyranose in the same buffer (PH7.0)were used as an enzyme and a substrate solution, respectively. The enzyme solution(1000ul) and 100ul of the test samples at various concentrations were mixed, and absorbance at 405nm was measured using a spectrophotometer (UV1800 Shimadzu, Japan). After incubation for 5 minutes,50ul of the substrate solution was added and incubated for an additional 5 minutes. The increase in absorbance from time zero was measured, and inhibitory activity was calculated as a percentage of the blank control. The % of inhibition was calculated using the following formula [15]

$$\text{Inhibition (\%)} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs 405 control}} \times 100$$

S.No	Concentration mg/ml	% of inhibition by Ascorbic acid (Standard)	% of inhibition by AgNps synthesized from Ethanolic flower extract	% of inhibition by AgNps synthesized from Petroleum Ether flower extract	% of inhibition by AgNps synthesized from Hydroalcoholic flower extract
1	0.2	22.69±0.23	4.17±0.85	12.50 ±0.63	18.33±0.06
2	0.4	30.36±0.33	16.67±0.54	20.83 ±0.23	20.83±0.15
3	0.6	45.87±0.57	20.83±0.69	29.17±0.12	33.33 ±0.21
4	0.8	69.03±0.70	33.33±0.63	33.33 ±0.85	31.36 ±0.58
5	1	84.98±0.39	45.83±0.47	37.50 ±0.47	45.83 ±0.30
IC 50 value		50.25 ɥg/ml	72.35ɥg/ml	79.23 ɥg/ml	60.05ɥg/ml



Conclusion

The enzymes, pancreatic alpha-amylase, and intestinal alpha glucosidase break down the oligosaccharides and disaccharides into mono-saccharides suitable for absorption [16]. The inhibition of these enzymes lowers the blood glucose level in blood [17]. The alpha-amylase and alpha-glucosidase were inhibited in a concentration-dependent manner followed by various concentrations of AgNps. The enzymatic activity was lowered remarkably when the concentration of AgNps was increased. The IC50 value of AgNps synthesized from Hydro alcoholic flower extract was higher both in alpha-amylase and alpha-glucosidase assays than other AgNps which were synthesized from Petroleum Ether and Ethanolic flower extract. The antioxidant and antidiabetic studies results proved that the Silver nanoparticles synthesized from Hydro alcoholic flower extract of *chrysanthemum indicum* can be used to reduce blood glucose levels and inflammation. It has notable applications in the pharmaceutical and biomedical industries.

Conflicts of interests

The authors declare that there is no conflict of interest.

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