Phytochemical Composition, Anti-oxidant, Anti-glycation, α-amylase and α-glucosidase Inhibition of Silver Nanoparticles from Extracts of Ardisia Solanacea and Cytotoxic Studies

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ISSN : 2382-5359(Online), 1994-1412(Print)

DOI:
https://doi.org/10.3126/njst.v22i2.78603

Date of Submission: 8 Feb, 2023

Date of Acceptance: 27 Dec, 2024

ABSTRACT

The aim of this work is to evaluate the anti-oxidant and anti-diabetic activity of extracts of Ardisia solanacea and assess the cytotoxicity as well. The leaves were subjected to solvent extraction and nanoparticles were synthesized using the extracts. The AgNP of methanol extract from leaves of A. solanacea showed the highest total phenolic and flavonoid contents of 35.44 mg of gallic acid/gm and 15.77 mg of quercetin /gm respectively. The TEM analysis revealed that the size of AgNPs ranged between 30 and 47 nm with λmax of 440nm. The AgNP of methanolic extract at 100µg/mL exhibited maximum activity of 89.6% and 84.9% for DPPH and H2O2 scavenging activities respectively. Further, the extracts exhibited excellent NO scavenging, Xanthine oxidase inhibition and anti-glycation activities. The AgNP of methanol extract displayed excellent inhibitory activity with IC50 of 45.47 µg/mL and 56.42 µg/mL respectively for α-amylase and α-glucosidase. The MTT assay for the cytotoxic studies in vitro of extracts on HepG2 cells at varied concentrations was carried out and all the tested extracts and compounds displayed low levels of toxicity in a dose-dependent approach. However, all the extracts exhibited <50% cell death even at the highest concentration of 200 µg/mL. Therefore, Ardisia solanacea could be explored as a bio-friendly natural source of phytoconstituents, which serves as an alternative to conventional synthetic compounds for the treatment and management of various diseases.

Keywords: Anti-oxidant activity, α-amylase, α-glucosidase, Ardisia solanacea, Cytotoxic activity and Silver Nanoparticles

1. INTRODUCTION

Diabetes mellitus is the most common disorder of insulin metabolism and is recognized as one of the health problems that need to be addressed on an urgent basis. According to the current data available in the Diabetes Atlas of the International Diabetes Federation (fourth edition), 285 million among the 7 billion of total world population are affected by diabetes mellitus (Wild et al, 2004). In addition, number of people suffering from impaired glucose tolerance are 344 million and numbers are expected to rise to 438 million for diabetes and 472 million for IGT by 2030 (IDF Diabetes Atlas 2021). Recent research depicts significant evidence stating that diabetes mellitus is a scourge in many low and middle-income countries. Almost one-fifth of the world's people in Southeast Asia are suffering from this disorder, which is responsible for many deaths in most of the countries, and caused 4.6 million deaths in 2011 (IDF Diabetes Atlas, 2021). According to the latest data from the World Health Organization published in 2016, the deaths in Sri Lanka due to diabetes mellitus are 5.7% of total deaths. Sri Lanka is a multi-etimic and multi-religious country with about 20 million people (WHO 2016). Suffering from diabetes is increasing globally with changes in urbanization, lifestyle changes and lack of physical activity. Diabetes cannot be cured as of now and however the blood glucose levels can be maintained through pharmacological and dietary interventions. Phytoconstituents from MAPs have excellent antioxidant and anti-glycation activities that prevent this auto-oxidation and help in preventing complications in diabetes. Over the last few decades, recognition of herbal treatments for diabetes has increased globally as they offer more efficacy, minimal side effects with affordable cost (Brusotti et al. 2014). There are several medicinal plants used in the herbal formulations of traditional medicine like Unani, Chinese and Ayurveda for the management of Diabetes mellitus, but few scientific experiments have proven the efficacy of the plants for their therapeutic potential in either prevention or management of Diabetes mellitus (Brusotti et al. 2014; Dhakad et al. 2018). Therefore, the cutting-edge research needs to evaluate the pharmacological potential and identify the active compounds, as these scientific findings helps the future remedy development industry, to prove the efficacy and standardize herbal medicines.

Medicinal plants and their products have been employed in the management of various metabolic disorders since time immemorial. Research on phytoconstituents from various botanicals showed that they regulate oxidative stress damage by scavenging the free radicals and reactive oxygen species (Ames et al. 1993; Pérez et al. 2021). Previous studies suggest that there is a correlation between disease incidence and diet where, the risk of occurrence of degenerative diseases is less with a higher intake of foods rich in antioxidants (Ghadermazi et al. 2017). Among these, are essential oils from medicinal and aromatic plants comprising mostly of volatile constituents with characteristic aroma and employed in drug, food and perfumery industries (Christenhusz & Byng 2016; Dhakad et al. 2018). There has been growing interest in the usage of plant extracts as anti-oxidant and anti-glycation agents thus conscientious efforts have been placed recently by many researchers throughout the world in the development of novel lead products for the maintenance of well being and management of diabetes.

Ardisia solanacea (A. solanacea) belonging to the family Myrsinacea is an evergreen shrub or tree growing to a height of 12 meters tall and distributed in tropical and subtropical regions of the world. It is commonly known as Duck's Eye in English, Bodina Gida in Kannada and Adavi Mayuri in Telugu. In Asia it is particularly distributed in Southern China, India, Sri Lanka, Nepal, Bhutan, Myanmar and Malaysia. The plant is a repertoire bioactive compounds and is widely used in the treatment of diarrhea, sore throat, dysmenorrhea, gout, rheumatic arthritis, vertigo, bacterial infections and thrombolytic diseases (Khatun et al. 2013; Amin et al. 2015). As a part of screening medicinal plants available in the Western Ghats, A. solanacea was selected based on the ethnobotanical information and literature and therefore, we here present our results of the investigative study.

2. MATERIALS AND METHODS

2.1 Chemicals, solvents, standard compounds and enzymes

The enzymes, α-amylase and α-glucosidase were purchased from Sigma-Aldrich, Bengaluru, India. The solvents and chemicals (analytical grade), Gallic acid, Ascorbic acid, Allopurinol, Aminoguanidine and Acarbose were procured from Sigma-Aldrich, India.

2.2 Plant material

The leaves and stems of Ardisia solanacea were collected near Korahalli (13°2'1.6728'' N 76° 4' 38.1864'' E), near Hassan, Karnataka, India in July 2021 and a voucher specimen has been deposited in the Department of Botany, AV Kanthamma College for Women, Hassan Karnataka (AVK BOT 3018).

2.3 Solvent extraction

The leaves and A. solanacea were subjected to solvent extraction by soaking them separately in various solvents such as Petroleum ether, Ethyl acetate, and Methanol for 8 h followed by homogenization in a mortar and pestle. Then the extracts were filtered using separating funnel, excess solvent was removed using a rotary evaporator under vacuum and dried over anhydrous Na_2SO_4. The light green and pale yellow extracts thus obtained were stored at 4 °C in a refrigerator for further studies.

2.4 Phytochemicals screening

The phytochemicals screening was carried out according to the qualitative standard chemical methods (Trease & Evans 2009). All the solvent extracts were screened for the presence of various phytochemicals such as carbohydrates, alkaloids, Terpenoids, Anthraquinones, Tannins, sterols, and flavonoids using various qualitative analytical tests.

2.5 Biosynthesis of Silver nanoparticles (AgNPs)

Freshly prepared extracts were used for the biosynthesis of AgNPs as per the method described (Parashar et al. 2009). A queous solution of silver nitrate (50 mL of 5×10^{-3} M) was prepared in an Erlenmeyer flask and 1 ml of each solvent extract (0.2 g/mL) was added and kept in the dark for 24 h until the appearance of a brownish color. The absorbance of silver nanoparticles was recorded using Sl-159 spectrophotometer at 200-800 nm range (Theerthavathy et al. 2019) followed by TEM analysis for determination of the size and shape of AgNPs formed (Elevazhagan & Arunachalam 2011).

2.6 Estimation of Total Phenolic content

This was performed as per published method (Ainsworth & Gillespie 2007) with slight modifications and in quintuplicate. Extracts and AgNPs (1 µg/mL; 100 µL), 1M Na_2CO_3 were mixed with Folin-Ciocalteau reagent and incubated in the dark for 15 minutes. The absorbance of the color developed was recorded at 760 nm against reagent blank. Gallic acid was employed as a positive control sample. The total phenolics were determined as mg of gallic acid equivalent per gm of extracts and samples.

2.7 Determination of Total Flavonoid content

The flavonoid content in the extracts and AgNPs was determined using colorimetric assay as per the published method (Aiyegoro et al. 2010) with slight modifications and in quintuplicate. Briefly, extracts (1ml), AgNPs (1mL), aluminum chloride (10%; 0.2 mL), potassium acetate (1M, 0.2 mL) and distilled water (5.6 mL) were mixed thoroughly and incubated at 37 °C for 30 min. Absorbance was recorded at 420 nm in a SL-159 spectrophotometer and the total flavonoids were determined as mg of quercetin equivalents per gram of sample from the calibration curve of quercetin.

2.8 In vitro Antioxidant activity

This was evaluated by adopting the following methodologies.

2.8.1 Determination of FRSA potential using DPPH

The Free Radical Scavenging Ability (FRSA) of extracts and AgNPs of extracts of A. solanacea determined using 1,1-diphenyl-2picrylhydrazyl (DPPH) radical scavenging method (Karadag et al. 2009) with slight modifications and in quintuplicate. The reaction mixture consisted of DPPH solution (1mmol/l in methanol, 2ml) and 1ml of extracts and Ascorbic acid (positive control) were incubated at 37°C for 20 min. The decrease in the absorbance was measured at 517 nm against a reagent blank. The FRSA potential of all samples was determined using the formula.

Free radical scavenging activity (%) = $(A_s - A_t) / A_s \times 100$

Where A_s = Absorbance of standard compound

A_t = Absorbance of sample

2.8.2 Determination of FRSA potential using H_2O_2

This was carried out as per the published method (Ruch et al. 1989) with slight modifications and in quintuplicate. Hydrogen peroxide (43 mM) in phosphate buffer (1 M, pH 7.4) was prepared and used for the analysis. Different concentrations of extracts (10-500µg/ml) were added to an H_2O_2 solution (0.6 mL, 43 mM), incubated for 10 min and absorbance was

measured at 230 nm against phosphate buffer (without H_2O_2) blank. The FRSA potential was assessed by the % inhibition using the formula

% inhibition = (Control- Test) /control ×100

2.8.3 β -Carotene bleaching assay

This assay was performed as per the method of Wettasinghe and Shahidi (1999) with slight modifications and in quintuplicate. Briefly, β -carotene (200µg/ml in chloroform; 2mL) was taken in a round bottom flask and added linoleic acid (20 µL) and Tween 20 (200 µL) mixed and vortexed and evaporated for 10 min at 40 °C. This was followed by the addition of 100 ml of distilled water (HPLC grade). Then the mixture was vortex and 5 ml of the resultant solution was transferred into test tubes containing varied concentrations (10-500 µg/mL) of extracts. Subsequently, the tubes were heated in a water bath at 50 °C for 2 h and then absorbance at 470 nm was measured every 15 min. The antioxidant activity was determined using the formula

Anti-oxidant activity % = $1 - (A_0 - A_t) / (A_0^0 - A_0^t)$

Where, A_0 = Absorbance of control; A_t = Absorbance of sample

2.9 Anti-glycation activity

The Anti-glycation assay was carried out as per the method of Bhatwadekar & Ghole (2005) with slight modifications and in quintuplicate. Briefly, 1 mg/mL of bovine serum albumin was incubated with fructose (0.25 M) and glucose (0.25 M) in phosphate-buffered saline (0.1M, pH 7.4) along with extracts and aminoguanidine (positive control) were dissolved in 50% DMSO and incubated in the dark for 4 days. The glycated protein thus formed was measured at two different wavelengths, 335 nm (Fluorescence, excitation wavelength) and 385 nm (emission wavelength). Extracts at varied concentrations ranging from 1- 100 µg/mL were tested and IC_{50} values were calculated. Subsequently, the percentage inhibition of AGEs was determined using the formula:

AGE % = $(F_{control} - F_{control\ blank}) \times 100 / (F_{extract} - F_{extract\ blank})$

Where,

$(F_{control} - F_{control\ blank})$ is the difference between the fluorescent intensity of BSA incubated with or without glucose and fructose.

$(F_{extract} - F_{extract\ blank})$ is the difference between the fluorescent intensity of BSA and sugars incubated with or without plant extracts.

2.10 α-Amylase inhibitory activity

The assay was performed as per the method of McCue and Shetty, 2004 with slight modifications and in quintuplicate. Extracts (10-100 µg/mL; 250 µL) were taken in suitable vials along with phosphate buffer (0.02 M; pH 6.9; 250 µL) consisting of **α**-amylase (0.5 mg/mL). The mixture was incubated for 10 min. at 37 °C. Starch at a concentration was employed as a substrate and added to the above mixture and further incubated for 10 min. Then the reaction was terminated by the addition of DNS (dinitro-salicylic acid) reagent (500 µL). Subsequently, the vials were placed in boiling water for 5 min, cooled and then diluted with water (5 mL). Subsequently absorbance was recorded at 540 nm. The mixture without extracts and Acarbose were served as negative control and positive control respectively. The enzyme inhibition percentage was measured using the following formula

% Inhibition = $\frac{A_{control} - A_{sample}}{A_{control}} \times 100$

2.11 α-Glucosidase inhibition assay

The α-glucosidase inhibition potential of extracts was evaluated according to the method of Kim et al. (2005) with slight modifications. p-Nitrophenylglucopyranoside (pNPG) (20 mM phosphate buffer, pH 6.9) was employed as a substrate. The **α**-glucosidase (1.0 U/mL, 100 µL) was first incubated with 50 µL of extracts of various concentrations (10-100 µg/mL) for 10 min followed by the addition of 50 µL of 3.0 mM pNPG and further incubated for 20 min at 37 °C. The reaction was terminated by using 2 mL of 0.1 M sodium carbonate. The p-nitrophenol released (yellow color) from pNPG was measured at 405 nm. The results were expressed as % blank control (background absorbance was corrected by replacing enzymes with buffer). Acarbose and buffer were employed as positive and negative controls respectively. The α-glucosidase inhibition percentage was determined by using the following formula

% Inhibition = $\frac{A_{control} - A_{sample}}{A_{control}} \times 100$

2.12 Cytotoxicity Assay

The cytotoxicity of the extracts was assessed according to the method of Mosmann (1983). The HepG2 cells were seeded into 96-well plates (100 µL, 8000 cells per well) and left overnight for adhesion process. Then 100 µl of each of the extracts at concentrations of 10-200

µl/ml/well were added and incubated for 48h at 37 °C. The worn-out medium was removed through aspiration. Subsequently, the DMEM medium (100 µL containing 10% fetal calf serum) and 0.5 mg/ml MTT were added and further incubated for 3h at room temperature. The MTT crystals from the medium were dissolved in DMSO (200 µL/well) and absorbance was recorded at 540 nm. The cytotoxicity was then measured as control % (medium only) and the cell death % and expressed using the equation.

$$\% \text{ Cell death} = 1 - \frac{Absorbance\ of\ test\ well}{Absorbance\ of\ untreated\ well} \times 100$$

2.13 Statistical Analysis

The IC_{50} values were determined by probit analysis. The data obtained was subjected to statistical analysis using GraphPad Prism version 8.01 (Graph pad software, Inc., La Jolla, CA, USA.) and Sigma plot (enzyme module) statistical software.

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening of extracts from leaves of A. solanacea showed the occurrence of alkaloids, terpenoids, flavonoids, tannins, carbohydrates and sterols (Table 1). The results showed that the addition of 0.2 g of the extract to 50 mL of 5 mM aqueous silver nitrate ($AgNO_3$) resulted in the formation of the brown solution after the overnight incubation at 40 °C in the dark. This indicates the formation of silver nanoparticles (AgNPs) that showed a maximum absorption at 440 nm (Fig-1). The TEM analysis revealed that the size of AgNPs ranged between 30 and 47 nm (Fig-2). The total phenolics and flavonoid contents were determined and expressed as mg of gallic acid per gm of sample and depicted in Table 2. The AgNP of methanol extract from leaves of A. solanacea showed the highest total phenolic and flavonoid contents of 35.44 mg of gallic acid/gm and 15.77 mg of quercetin /gm respectively. While, it was 33.71 mg of gallic acid/gm and 13.08 mg of quercetin / gm respectively in the crude methanol extract.

3.1 Anti-oxidant activity

The antioxidant efficacy of extracts of A. solanacea was evaluated and results were depicted in Tables 3-5. The AgNP methanolic extract at 100µg/ml showed maximum activity of 89.6% and 84.9% for DPPH and H_2O_2 scavenging activities respectively. Ascorbic acid exhibited 100% activity at 80µg/ml in both the assays. These results indicate that AgNPs methanol extract has excellent antioxidant activity and is comparable with that of ascorbic acid. The results of β-carotene bleaching assay revealed good antioxidant activity of AgNPs methanol extract with IC_{50} of 58.19 µg/mL followed by methanol extract (IC_{50} of 64.81 µg/mL), ethyl acetate (IC_{50} of 73.34 µg/mL) and pet ether extracts (IC_{50} of 87.28 µg/mL). With the increase in concentration of extracts, a subtle but noticeable increase in the activities was observed in a dose-dependent manner.

3.2 NO scavenging activity

The NO scavenging ability of all the samples was evaluated in a dose-dependent manner using gallic acid as a positive control sample and the results were presented in Table 6. Among all the samples tested, AgNP methanol extract exhibited potent NO scavenging activity with IC_{50} of 37.41 µg/mL followed by methanol (48.12 µg/mL), Ethyl acetate (57.19 µg/mL) and pet ether (76.62 µg/mL) extracts. However, the positive control gallic acid showed an IC_{50} of 29.53 µg/mL. The percentage inhibition with an increase in concentration of extracts and gallic acid was evaluated and the significant observation was that with an increase in concentration, a notable increase in percentage inhibition of NO scavenging activity was observed.

3.3 Xanthine oxidase inhibition

All the extracts were evaluated as potential XO inhibitors and compared favorably with Allopurinol, a positive control and the results are summarized in Table 7. Among all the extracts tested, AgNP methanol extract showed maximum xanthine oxidase inhibition activity with an IC_{50} of 28.78 µg/mL. The methanol, ethyl acetate, and pet ether extract also demonstrated excellent xanthine oxidase inhibition with an IC_{50} of 38.13, 60.08, and 72.18 µg/mL respectively. While, Allopurinol showed an IC_{50} of 18.17 µg/mL.

3.4 Anti-glycation activity

The ability of A. solanacea extracts to inhibit advanced glycation end product (AGE) formation was evaluated and presented. In the assay, bovine serum albumin, glucose and fructose served as the model protein and glycating agents respectively. The formation of AGEs was evaluated by monitoring the production of fluorescent products formed at 335 nm and 385 nm, respectively, and the results are depicted in Table 8 and all the extracts inhibited AGE formation in a

dose-dependent manner. The AgNP methanol extract presented a higher inhibitory effect of more than 90% inhibition of AGE formation at a concentration of 100 µg/mL with an IC_{50} of 35.12 µg/mL. Methanol extract also displayed excellent anti-glycation activity with an IC_{50} value of 44.28 µg/ml followed by Ethyl acetate (64.13 µg/mL) and Pet ether (72.29 µg/mL) extracts. While, the Aminoguanidine, showed IC_{50} of 22.76 µg/mL. The degree of activity was evaluated for all the extracts and compounds at a concentration ranging from 1-100 µg/mL where, increase in percentage inhibition with increase in concentration was observed.

3.5 α- Amylase inhibition

The α-amylase enzyme inhibition of extracts was evaluated and presented in Table 9. The AgNP methanol extracts demonstrated excellent inhibitory activity with IC_{50} of 45.47 µg/mL while Acarbose showed an IC_{50} value of 28.49 µg/mL. The methanol and ethyl acetate extract also showed good inhibition with an IC_{50} of 59.23 and 66.05 µg/mL respectively. The assay was performed at varied concentrations of 0-100 µg/mL and with increase in concentration a noticeable increase in the inhibition percentage was noticed (Fig. 3).

3.6 α-Glucosidase inhibition

The α-glucosidase enzyme inhibition of extracts was evaluated and presented in Table 10. AgNP methanol extract displayed excellent inhibitory activity with IC_{50} of 56.42 µg/mL while it was 33.51 µg/mL for acarbose. Both methanol and ethyl acetate extracts showed good inhibition with IC_{50} of 61.71 and 77.27 µg/mL respectively. While, pet ether extract demonstrated mild inhibition with IC_{50} of 88.61 µg/mL. The assay was performed at varied concentrations of 0-100 µg/mL where, increase in the inhibition percentage was noticed with increase in the concentration of samples (Fig. 4).

3.7 Cytotoxicity studies

The assay employed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) for evaluation of cytotoxicity of all the extracts on HepG2 cells at varied concentrations (0-200 µg/mL). All the tested extracts presented low level of toxicity at all the concentrations in a dose-dependent manner and the results are depicted in Fig. 5-8. The IC_{50} values obtained were 458.79, 358.53, 292.28 and 241.72 µg/mL respectively, for AgNP methanol extract, methanol, ethyl acetate and pet ether extracts. The significant feature was that all the extracts exhibited <40% cell death even at the highest concentration of 200 µg/mL.

4. DISCUSSION

The phenols, flavonoids, and terpenes of various medicinal plants are well known important compounds in plants contributing to the antioxidant potential of various botanicals (Khan et al. 2019; Batool et al. 2019; Bindu et al. 2020). Phytochemicals derived from medicinal botanicals are known to possess primary antioxidant activity as they can react with active oxygen radicals, such as hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals and inhibit lipid oxidation at an early stage. It has also been reported that the increase in the phenolic content can be used as a biomarker for screening of air pollutants (Howell 1974; Stankovic et al. 2016). Considerable research has been carried out and established that flavonoids are antioxidants compounds and recognized as valuable nutraceuticals for neutralizing free radical stress (Gunathilake et al. 2018). The antioxidant properties of plant extracts can often be attributed to the presence of substantial amount of phenolics and flavonoids. In the present study, all the extracts from A. solanacea demonstrated excellent anti-oxidant activity and this can be correlated to the presence of more amounts of phenolics and flavonoids and might be employed in the treatment of stress and other related disorders.

The phenolics, flavonoids and other phytochemicals derived from medicinal plants have known to act as xanthine oxidase inhibitory agents (Cho et al. 2008; Shukor et al. 2018). The Plants are well known as a rich source of polyphenols and some of the common species, were previously reported to possess XO inhibitory effects (Cho et al. 2008; Shukor et al. 2018). Moreover, the results in the present study are in agreement with those of previously reported plants (Grevsen et al. 2009; Hudaib et al. 2011). At present, Allopurinol is the drug of choice for gout disease and people are using it more frequently. However, upon prolonged usage, the drug presents various side effects. Therefore, as a part of screening program of medicinal plants of Western Ghats, South India, and also based on the ethnobotanical information, A. solanacea plant was selected for xanthine oxidase inhibitor activity evaluation, wherein, all the extracts have displayed marked inhibitory activity comparable to allopurinol.

The effect of plant-derived compounds and extracts

on protein glycation was evaluated by the inhibition of fructosamine formation. It was found that all the extracts of A. solanacea exhibited varied degree of potential to inhibit initial stages of glycation reaction. The hyperglycemic state that is commonly seen in Diabetes mellitus patients was reported to be associated with cardiovascular complications (Chen et al. 2011) and this evidence implicates the formation and subsequent effects of AGEs as a contributing cause for such a complication. Further, it has been reported recently that there is increased oxidative damage in the vicinity of glycated histone residues (Guedes et al. 2011; Mahomoodally et al. 2019). Advanced glycation end products (AGEs) have been involved in the pathogenesis of diabetes and aging-related complications and therefore, inhibition of glycation should have a broad and beneficial effect in the treatment. However, several traditionally used herbal medicines and essential oils have been shown to possess in vitro anti-glycation effects due to the presence of flavonoids, terpenes and phenols (Mahomoodally et al. 2019). The AgNP extract in the present study displayed excellent ant-glycation activity by inhibiting more than 90% of AGEs formation which can be attributed to the presence of higher concentration phenolics and flavonoids. However, the synergistic effect of all the components in the extract cannot be neglected.

Plants are the major source of valuable biochemicals and bioactive compounds for the development of lead products. The extracts of A. solanacea was reported to exhibit good antioxidant, antimicrobial, thrombolytic, anthelmintic, insect antifeedant and cytotoxic activities (Samal 2013; Amin et al. 2015; Anjum et al. 2019; Islam et al. 2019; Pournami and Pratap Chandran, 2021). But the anti-oxidant and anti-glycation potential of silver nanoparticles of the extract and inhibition of α-amylase and α-glucosidase was not reported. The novelty of the present work lies in the exploration of potential of silver nanoparticles of the extracts of the selected plant to inhibit the formation of AGEs and carbohydrate metabolizing enzymes.

The use of medicinal plants in the management of diabetes has been followed and many therapeutic plants with anti-diabetic activity were reported in the literature (Saeedi et al., 2019; Nipun et al., 2021). Several reports have been focused on the studies of the therapeutic potential of medicinal plants and phytoconstituents in alleviating diabetes by inhibitory effects against α-amylase and α-glucosidase (Loodu & Rupasinghe 2019). The molecules acting as α-amylase and α-glucosidase enzyme inhibitors are recognized and portrayed to delay the breakdown of carbohydrates in the small intestine and decrease the postprandial blood glucose levels (Assefa et al., 2020). This feature is tapped and considered as one of the key strategies for the maintenance of blood glucose levels. The results obtained from this study showed that these extracts possess promising α-amylase and α-glucosidase enzyme inhibition, which are in agreement with the published reports (Assefa et al. 2020). However, identification and evaluation of toxicity of the phytoconstituents and botanical extracts is indispensable before making it a formulation and incorporating them into traditional medicine. The cytotoxicity of extracts was assessed on HepG2 cells and the results reveals that all the samples exhibited less than 40% cell death even at the highest concentration of 200 µg/ml. All the samples displayed a very low level of toxicity in comparison with the plants described and published in the literature which have been presented with > 50% cell death (Kuete et al. 2017). This particular aspect encourages for the use of this plant and extracts in herbal formulations for the treatment of various ailments.

5. CONCLUSION

The current study revealed that silver nanoparticles can be synthesized in a simple method using A. solanacea leaf extract. The TEM analysis showed that the sizes of the synthesized AgNps ranged from 30 to 50 nm. The AgNP and other extracts showed excellent anti-oxidant activity, NO scavenging activity and xanthine oxidase inhibition and this can be attributed to the presence of a substantial amount of phenols, flavonoids, and terpenoids. Advanced glycation end products (AGEs) have been involved in the pathogenesis of diabetes and aging-related complications and therefore, inhibition of glycation should have a broad and beneficial effect in the treatment. The AgNPs and other extracts in the present study displayed excellent ant-glycation activity with >90% inhibition of AGEs. The MTT assay for the cytotoxic studies of extracts on HepG2 cells at varied concentrations was carried out and all the tested extracts displayed low levels of toxicity at all concentrations in a dose-dependent approach. However, the extracts exhibited <50% cell death even at the highest concentration of 200 µg/mL. Therefore, further studies are underway to isolate phytoconstituents of this plant and other biochemical aspects where the data procured might help in the lead products and formulation development for the management of stress-related

disorders and diabetes.

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr. J. Achyutha Devi, Principal, R.B.V.R.R. Women's College, Narayanaguda, Hyderabad, India for providing the necessary facilities to carry out the work and also for her encouragement and support.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no conflict of interest.

Table 1: Phytochemical screening of extracts of A. solanacea

Parts used	Secondary metabolites	Petroleum ether extracts	Ethyl acetate extracts	Methanol extracts	AgNP extract
Leaves	Tannins	-	+	+	+
	Flavonoids	-	-	+	+
	Alkaloids	-	+	++	++
	Terpenoids	+	+	++	++
	Anthaquinones	_	_	-	-
	Carbohydrates	_	+	+	+
	Sterols	++	+	+	+

Data shown as obtained from the analysis (n=5)

Table 2: Total phenolics and flavonoid content in the extracts of A. solanacea

Extract	Total phenolics content (mg of gallic acid/gm)	Total flavonoid content
Methanol extract	33.71±0.77	13.08± 0.59
Ethyl acetate extract	28.62±0.98	10.26± 051
Pet ether extract	22.26±1.06	8.17± 0.99
AgNP of Methanol extract	35.44±1.23	15.77± 1.27

Data presented as Mean ± SEM

Table 3: DPPH anti-oxidant scavenging activity of extracts of A. solanacea

Concentration (µg/ml)	DPPH radical scavenging activity (%)				
	Pet ether extract	Ethyl acetate Extract	Methanol	AgNP Methanol extract	Ascorbic acid
10	6.73 ± 0.61	9.71 ± 0.43	11.12 ± 0.79	14.72 ± 0.98	21.23±0.57
20	12.11 ± 0.84	17.34 ± 0.92	22.25 ± 0.92	27.21 ± 0.89	33.45 ± 0.98
40	23.08 ± 1.06	28.38 ± 1.02	34.18 ± 1.14	40.08 ± 1.01	48.19 ±1.17
60	35.76 ± 1.22	43.67 ± 1.15	46.38 ± 1.62	58.47 ± 1.23	78.26 ± 1.28
80	44.93 ± 1.99	54.18 ± 1.98	64.57 ± 2.26	67.19 ± 2.17	100
100	57.28 ± 2.25	68.26 ± 2.07	78.72 ± 2.48	89.61± 1.24	100

Test extracts: significant from normal control, $P < 0.05$
Values presented as Mean ± SEM

Table 4: H_2O_2 free radical scavenging activity of extracts of A. solanacea

Concentration (µg/mL)	Hydrogen peroxide free radical scavenging activity (%)				
	Pet ether extract	Ethyl acetate Extract	Methanol	AgNP Methanol extract	Ascorbic acid
10	5.35 ± 0.52	9.17 ± 0.37	9.04 ± 0.68	11.22 ± 0.72	24.45±0.71
20	9.11 ± 0.79	18.34 ± 0.82	19.65 ± 0.93	23.16± 0.93	38.51 ± 0.96
40	17.15 ± 1.12	26.26 ± 1.22	30.23 ± 1.23	38.28 ± 1.21	51.32 ±1.08
60	29.51 ± 1.38	40.67 ± 1.35	48.19 ± 1.75	65.81 ± 1.25	88.16 ± 1.37
80	36.34 ± 1.91	51.23 ± 1.78	60.39 ± 2.29	73.37± 1.09	100
100	52.81 ± 2.34	64.16 ± 2.37	72.28 ± 2.37	84.92± 2.19	100

Test extracts: significant from normal control, $P < 0.05$

Values presented as Mean ± SEM

Table 5: β-carotene bleaching activity of extracts of A. solanacea

Sl. No.	Sample/Extract	IC50 (µg/mL)
1.	Pet ether extract	87.28 ± 2.15 (72.288 – 92.193)
2.	Ethyl acetate extract	73.34 ± 1.56 (63.724 – 81.156)
3.	Methanol extract	64.81 ± 1.32 (54.189 – 69.342)
4.	AgNP extract	58.19 ± 1.27 (49.211 – 64.394)

Test extracts: significant from normal control, $P < 0.05$Values presented as Mean ± SEM

Table 6: NO scavenging activity of essential oil, extracts and isolated compounds from leaves of A. solanacea

S. No.	Sample/Extract	IC50 (µg/mL)	Relative Potency
1.	Pet ether extract	76.62 ± 1.58 (69.198 – 92.232)	0.385
2.	Ethyl acetate extract	57.19 ± 1.61 (65.183 – 85.412)	0.516
3.	Methanol extract	48.12 ± 1.42 (56.721 – 72.328)	0.614
4.	AgNP Methanol extract	37.41 ± 1.49 (28.188 – 46.237)	0.789
8.	Gallic acid	29.53 ± 1.31 (20.172 – 30.244)	1

Test extracts: significant from normal control, $P < 0.05$
Values are expressed as Mean ± SE; Relative potency = IC_{50} standard/IC_{50} sample
Numbers in parenthesis represent 95% confidence limits

Table 7: Xanthine oxidase inhibitory potential of extracts of A. solanacea

Compound	IC50 (µg/mL)	Relative Potency
Pet ether extract	72.18 (62.244 – 84.282)	0.252
Ethyl acetate extract	60.08 (57.281 – 66.402)	0.302
Methanol Extract	38.13 (27.228 – 45.417)	0.476
AgNP methanol extract	28.78 (22.627 – 36.608)	0.631
Allopurinol	18.17 (13.621 – 23.893)	1

Test extracts: significant from normal control, $P < 0.05$; All values are expressed as Mean ± SE; Relative potency = IC_{50} standard/IC_{50} sample;
The numbers in parenthesis represents 95% confidence limits

Table 8: Anti-glycation activity of extracts of A. solanacea

Compound	IC50 (µg/mL)	Relative Potency
Pet ether extract	72.29 (66.991 – 89.407)	0.315
Ethyl acetate extract	64.13 (54.812 – 76.245)	0.354
Methanol extract	44.28 (34.138 – 54.188)	0.514
AgNP methanol extract	35.12 (26.231 – 41.463)	0.648
Aminoguanidine	22.76 (18.231 – 27.334)	1

Test extracts: significant from normal control, $P < 0.05$

All values are expressed as Mean ± SE; Relative potency = IC_{50} standard/IC_{50} sample;

The numbers in parenthesis represents 95% confidence limits

Table 9: α-amylase inhibition of extracts from A. solanacea

Compound/Extract	IC50 value (µg/ml)	Relative Potency
Petroleum ether extract	83.32 ± 3.12	0.342
Ethyl acetate extract	66.05 ± 4.01	0.431
Methanol extract	59.23 ± 2.98	0.481
AgNP methanol extract	45.47 ± 2.43	0.627
Acarbose	28.49 ± 0.42	1

Values shown as mean ±SE (P<0.05)

Table 10: α-glucosidase inhibition of extracts from A. solanacea

Compound/Extract	IC50 value (µg/mL)	Relative Potency
Pet ether extract	88.61 ± 2.33	0.366
Ethyl acetate extract	77.27 ± 2.17	0.419
Methanol extract	61.71 ± 2.29	0.525
AgNP methanol extract	56.42 ± 2.12	0.575
Acarbose	33.51 ± 1.18	1

Values presented as mean ±SE (P<0.05)

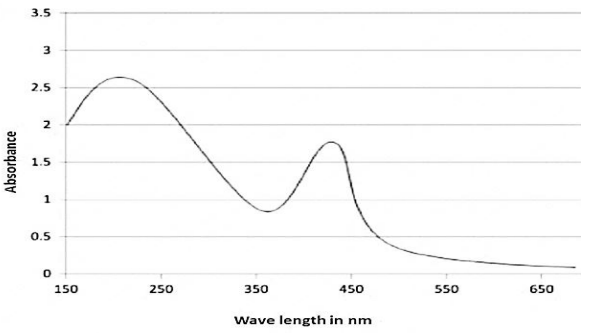

Fig.1: UV Absorption curve of AgNPs of methanol extract

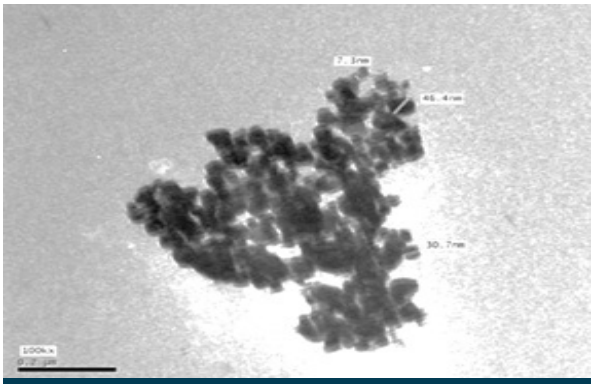

Fig.2: TEM of AgNPs of methanol extract of A. solanacea

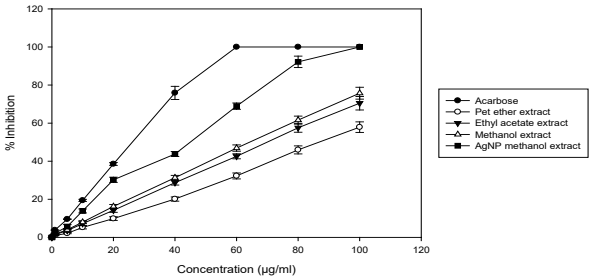

Fig.3: Inhibition (%) of α-amylase activity at varying concentrations of extracts from A. solanacea. Values presented as mean ±SE (P<0.05)

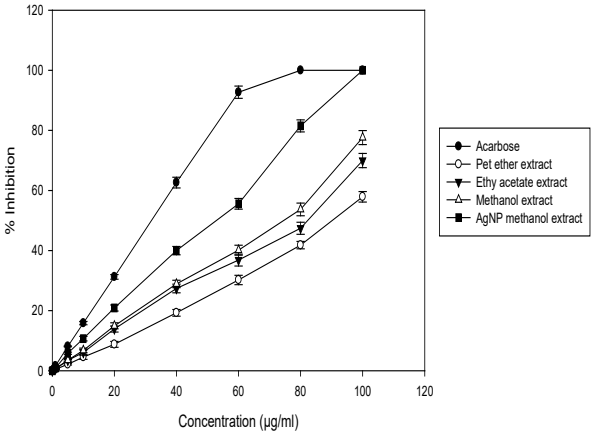

Fig.4: Inhibition (%) of α-glucosidase activity at varying concentrations of extracts from A. solanacea.

Values presented as mean ±SE (P<0.05)

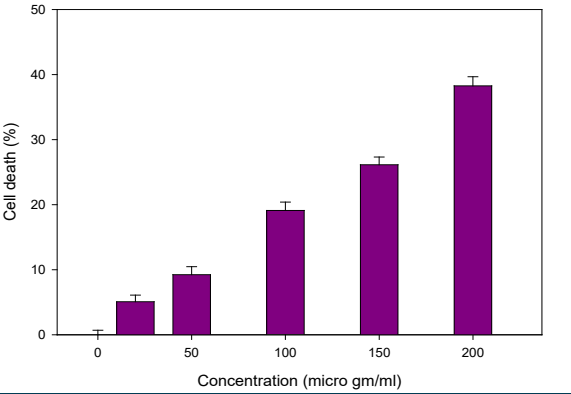

Fig.5: Cytotoxicity assay of the petroleum ether extract of A. solanacea HepG2 liver cells. Data presented as % control ± SE (p<0.05)

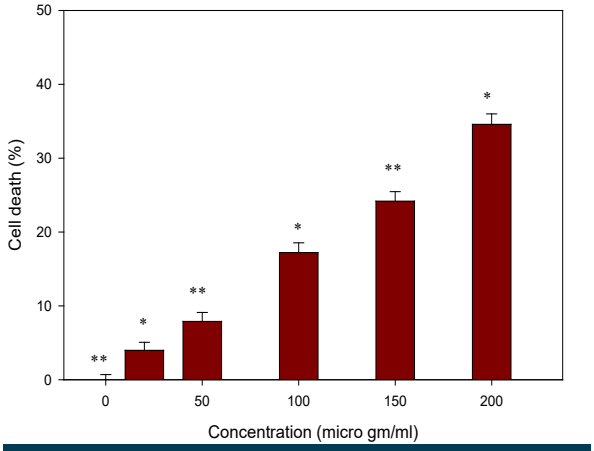

*Fig.6: Cytotoxicity assay of the Ethyl acetate extract of E. monogynum on HepG2 liver cells. Data presented as % control ± SE (*p<0.05; **p<0.01)*

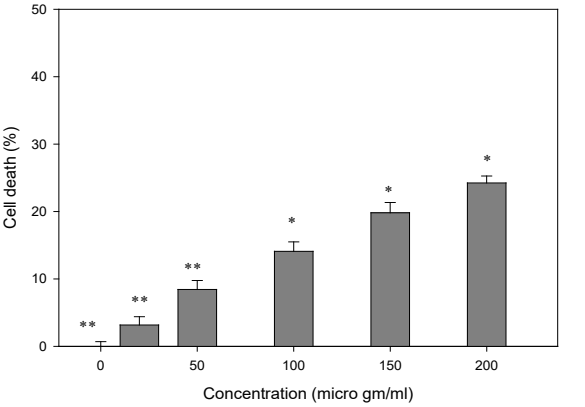

*Fig.7: MTT cytotoxicity of the Methanol extract from leaves of A. solanacea on HepG2 liver cells. Data presented as % control ± SE (*p<0.05; **p<0.01)*

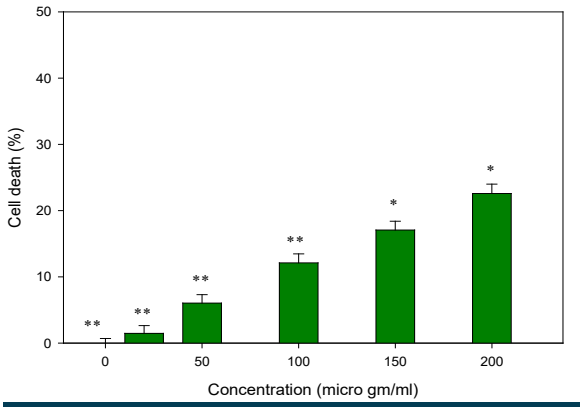

*Fig.8: MTT Cytotoxicity of the AgNP extract from leaves of A. solanacea on HepG2 liver cells. Data presented as % control ± SE (*p<0.05; **p<0.01)*

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