Antidiabetic potential and high synergistic antibacterial activity of silver nanoparticles synthesised with Musa Paradisiaca tepal extract

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ABSTRACT
This work investigates the Musa Paradisiaca plant and its tepal extracts. The research findings show that the tepal extracts of M. Paradisiaca contain high phytochemical activity. Hence we can conclude that these plants have a number of beneficial properties. Phytochemical analysis concludes that the plant is rich in flavonoids, phenolic compounds, tannins, terpenoids, and phytosterol. In the current work, silver nanoparticles (AgNPs) have revealed the antioxidant properties of M. Paradisiaca. The results show that the methanolic extracts of these tepals exhibit antioxidant potential and are also sources of natural antioxidant compounds, though comparatively, AgNPs have shown the best antioxidant activity. This work investigates the link between the ethnomedicinal and the bioactive constituents found in M. Paradisiaca toward all probable markers for cervical cancer via in vivo studies and molecular docking, to form a pharmacophore setting for the active target. However, most of the mechanisms of action of herbal medicines are not in total agreement, and the information collected from their traditional remedies over the years must not be neglected. Hence, it is sensible to investigate the options available in herbal medicine for cancer progression. Biosynthesised AgNPs are principally spherical and nanosized. It was also found that tepal-mediated AgNPs exhibit excellent antimicrobial efficacy against tested human pathogens. This green method can be used as a better alternative source than the chemical fabrication of nanomaterials and the biosynthesised nanoparticles can be used in antibacterial medicines. The methanolic tepal extract of M. Paradisiaca with AgNPs displayed proficient antidiabetic properties in the diabetes rat model and so could have a possible development for medical use in the future.

KEYWORDS:
Musa Paradisiaca, diabetes, antioxidant, silver nanoparticles, flavonoids

INTRODUCTION
Nanotechnology is a rapidly growing field owing to its application in science and technology for engineering new materials at the nanoscale level. The term “nano” specifies one billionth of a meter (10−9) and particles of size less than 100 nm are stated as nanoparticles (NPs). Currently, nanotechnology is one of the key compelling research areas in material science with the discovery of different plants for the use of NP synthesis. Nanotechnology can be administered in various fields including agriculture, medicine, healthcare, transport, textiles, water treatment, and cosmetics, which enhances the value of products. It is a characterisation, design, production, and application system by monitoring the shape and size on a certain nanometer scale. Diabetes mellitus (DM) is an endocrine metabolic disorder and it is quickly cumulating worldwide. People with DM are not able to secrete sufficient insulin, hence they have abnormal levels of blood glucose in their body. If this is not treated, it may lead to severe disease. The most common form of the disease is differentiated into two main types: Type-1 and Type-2 DM. Type-1 is responsible for at least 5%–10% of the total cases while Type-2 comprises more than 90%–95% of all cases. The risk factors of DM are undefined, however both genetic and environmental factors including obesity and lack of physical activity seem to play an important role.

DM-associated complications include diabetic retinopathy, diabetic nephropathy, and arteriosclerosis, which are the principal causes of morbidity and mortality among diabetic patients. These complications are caused by low levels of cellular antioxidant enzymes which lead to oxidative stress. The World Health Organization (WHO) reports that patients with diabetes are predicted to grow to 300 million or even more by the year 2025. Studies of diabetes has grown recently and its treatment options are therapies including use of insulin, medicinal plants, and numerous other oral antidiabetic drugs such as sulfonylureas, biguanides, alpha-glucosidase inhibitors, and glinides. It has been seen that entirely oral antidiabetic agents have adverse effects.

In the literature, there are 400 plant species mentioned that have hypoglycemic properties. However, the search for new antidiabetic agents from medicinal plants continues to be in demand because they contain novel substances that reveal safe and cost-effective impacts on DM. Most natural plants...
have secondary metabolites such as flavonoids, alkaloids, glycosides, terpenoids, and carotenoids that are commonly associated with antidiabetic activity. Ethnobotanical evidence recommends that about 800 plants may contain antidiabetic potential, and among them, Momordica charantia, Pterocarpus marsupium, and Trigonella foenum greacum seem to be most valuable for the treatment of Type 2 diabetes.

Medicinal plants have an optimistic future as there are still half a million undiscovered plants in the world. However, the application of the whole plant for research or treatment has many disadvantages, such as variations in the plant bioactive components based on the climate. The different plant parts are the root, fruit, skin, leaf, seeds, and flowers. The bioactive materials in almost all medicinal plants have treatment properties that can serve as therapeutic agents. There are over one-tenth of plant extracts (more than 50,000 species) that are incorporated into cosmetic and pharmaceutical products. Plants with antioxidant properties have been reported to be valuable for atherosclerosis and cardiovascular disease prevention by halting lipids peroxidation. Natural products have various mechanisms to cope with this issue, using antioxidant enzymes.

NPs from plants are much stable and the synthesis is more rapid than NPs from microorganisms. The benefits of using medicinal plant and plant based compounds for the synthesis of metal NPs have been attentive to explore the mechanisms of metal ions uptake and bioreduction by the plants, and the likely mechanisms of metal NPs production in plants. The plant extracts may function as reducing and capping agents in NPs synthesis. NPs can be used to monitor the oxidative status of tissues in vivo using conjugated biosensor. NPs are reported to have antimicrobial activity. They are used for cell tagging and drug delivery, and can also be applied as a conjugated biosensor for reaction oxygen species in diabetes. Nanotechnology is concerned with the synthesis of NPs of various sizes and shapes and their potential applications.

The use of medicinal remedies for the prevention and treatment for the disease is of increasing because the advantage and efficiency phytoconstituents activity in the herbs. Medicinal plants have a number of medicinal. One of such plant is M. paradisiaca. It has been reported to have biological activities such as antioxidant, antilithiatic, antimicrobial, antidiabetic, anti diarrhoeal, anti ulcer, hepatoprotective, hypcholesterolaemic, anti-snake venom, wound healing and anti menorrhagic. Banana is the commonly used term used for genus Musa, herbaceous plants for the fruit they produce. It is one of the oldest cultivated plants. All the parts in banana tree have medicinal uses: the flowers are used in dysentery and bronchitis and ulcers. Cooked flowers used in patients with diabetes. The astringent plant is used for patients such as hysteria, leprosy, fevers, epilepsy, haemorrhages, acute dysentery and diarrhoea and also for hemorrhoids, insect bites, and stings. In this study M. paradisiaca flowers (tepals) the edible part will be used for anti diabetic activity with the help of NPs. In previous studies antidiabetic drugs were discovered from different plant materials which will be used on human kind. The key objective of the study is to determine the ethno pharmacological knowledge of M. paradisiaca using green technology.

MATERIALS AND METHODS
Collection of specimens
The M. paradisiaca flowers were collected from vegetable market of Koyambedu, Chennai. The plants were washed properly with tap water and air-dried. The drying of plant is necessary to eliminate the water remaining from the plants before storing. The air dried plants were powdered using conventional blender for future use.

Preparation of methanolic crude extract
Compound with limited solubility in solvent must be subjected to soxhlet extraction. This method uses only single batch of solvent instead of several portions of solvent being exposed to the compound. However, this method cannot be used for thermo labile compound as extended exposure to heating can lead to degradation of compounds.

Qualitative analysis of phytocomponents
Phytochemical analysis was performed for the following components such as alkaloids, amino acids, carbohydrates, phenolic compounds, saponins, phytosterols, flavonoids, and terpenoids.

Synthesis of silver nanoparticles
An amount of 1 mM silver nitrate (AgNO₃) in aqueous solution was prepared in 250 mL. For reduction into Ag⁺ ions, tepal extract was added. The mixture was microwaved at 300 W for 4 min for complete bioreduction to avoid pressure variation. After cooling, the mixture was stored in air for 24 h for comprehensive bioreduction. The silver nanoparticle (AgNP) formation was confirmed by spectrophotometric analysis.

Characterization of AgNPs
Analysis using scanning electron microscopy (SEM) was performed by fabrication of suspension onto clean electric stubs and permitting water for complete evaporation. For voltage acceleration to 15 kV, JEOL-5800-LV16 SEM was used with a sample current of 41 μA.

Antioxidant assay (DPPH Assay)
The crude extracts and AgNP antioxidant effects were evaluated by DPPH assay according to the method described previously. The free radical DPPH (2,2-diphenyl-2-picryl hydrazy1) changes to diphenyl–picryl hydrazine which is noticed by color changes from deep violet to light yellow when acted upon by an antioxidant. The changes can be measured using a spectrophotometer at 518 nm to determine.
the DPPH scavenging activity of the antioxidant sample. Extracts measuring 25 μm and 0.48 ml of methanol were added to 0.5 ml of methanolic solution of DPPH. The mixed solution was allowed for reaction to take place at room temperature for at least 30 min. Methanol was used as blank and AgNPs served as the positive control. After incubation, the purple colored discolorisation was measured at 518 nm.

**Antibacterial activity by disc diffusion method**

Antibacterial activities of crude extracts and AgNPs were carried out using disc diffusion technique following the Kirby–Bauer technique.25,26 The bacterial strain was culture in nutrient agar (NA). Pure culture was inoculated from the Petri plate to the MHA plate for subculture at 37°C for 24 h. The aseptically prepared inoculum was prepared by inoculating the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density (1.5×108 cfu/ml) was adjusted according to 0.5 McFarland turbidity standard. Then the inoculum was plated evenly on an MHA plate to yield a lawn culture. Sterile Whatman No. 1 filter paper discs 5 mm in diameter were placed and impregnated with plant extracts of inoculum was plated evenly on an MHA plate to yield a lawn culture. Sterile Whatman No. 1 filter paper discs 5 mm in diameter were placed and impregnated with plant extracts. A clear zone around the disc, indicating inhibition of bacterial growth. The following day, the plates were monitored for a clear zone allowed to incubate for 24 h at 4ºC and incubated at 37ºC. The diameter were placed and impregnated with plant extracts (100 μg/disc) on the inoculated MHA plates. The plates were incubated at 37ºC for 24 h. The aseptically prepared inoculum was prepared by inoculating the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density (1.5×108 cfu/ml) was adjusted according to 0.5 McFarland turbidity standard. Then the inoculum was plated evenly on an MHA plate to yield a lawn culture. Sterile Whatman No. 1 filter paper discs 5 mm in diameter were placed and impregnated with plant extracts.

**Characterisation of crude extract and AgNPs by SEM**

The morphology of the produced AgNPs using SEM magnified at 9400X and 10,000X are shown in Figure 2 for both the control and treated samples. The monodispersed spherical AgNPs were shaped on the surface of methanolic crude extract derived biological materials. The picture acquired from FESEM also presented spherical NPs, approving the result achieved by SEM.

**RESULTS**

**Methanolic crude extract of *M. Paradisiaca***

The methanolic crude extract was prepared from the powdered flowers of *M. paradisiaca* using soxhlet apparatus at 1.4 mg after yield. The extract was concentrated by vacuum and dried in a dessicator.

**Phytochemical screening of methanolic extract of *M. paradisiaca***

Qualitative analysis of the methanolic crude extract revealed the existence of flavonoids, alkaloids, glycosides, polyphenols, tannins, proteins, saponins, sterols, and triterpenes in the tepal extract (Table I).

**Synthesis of AgNPs**

Noble metals are recognized to display exclusive optical activity as they have the property of surface plasmon resonance. The AgNPs formation was supervised with change of color. The color of the reaction mixture changed to yellowish brown in 10 min and to reddish brown after 1 h, signifying the production of AgNPs, because silver metal ions Ag⁺ reduced to AgNPs Ag through the active molecules.

**Characterisation of crude extract and AgNPs by SEM analysis**

SEM images of the crude extract and AgNPs are shown in Figure 1. It is observed that different shapes of AgNPs were taken from different tepal extracts and used as reducing and capping agents. *M. paradisiaca* tepal extracts produced are spherical, triangular, and cuboidal AgNPs. This might be affected by the different quantity and nature of capping agents in the different tepal extracts.

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**Cell viability assay**

The cytotoxicity and cell viability were assessed by colorimetric assay (MTT). Cells were cultured (1X105 cells/mL) in culture plates (96 well) and pretreatment was done according to the assay requirements. In all, 10 μm of MTT (5 mg/mL) was supplemented to the cell containing well and subjected to incubation for another 4 h. The solution was decanted and the tetrazolium product was dissolved with 100 μL of DMSO per well. The absorbance reading was obtained (570 nm) using a spectrophotometer and the cell viability values were calculated.27
Antioxidant activity

DPHH assay

The percentage inhibition of free radical generation by methanolic extracts was found to increase in a concentration-dependent manner, showing an IC50 value of 152.6 μg/ml (33.4% inhibition) and 118.2 μg/ml (59.36% inhibition), respectively, compared with the standard, ascorbic acid IC50 value (112.7 μg/ml). With regard to peroxide radical activity, MFF extract exhibited high inhibition (58.63% with IC50 value of 130.3 μg/ml) compared to TTF (53.26% inhibition with IC50 value of 190.4 μg/ml) when taking into account the IC50 value of standard, ascorbic acid (111.7 μg/ml) (Figure 2).

Disc diffusion method

The methanolic extract antibacterial activity against human pathogenic organisms by disc diffusion technique demonstrated a full zone of inhibition. The antibacterial activity of AgNPs was examined on Bacillus, Pseudomonas, Staphylococcus, and E. coli colonies in NA plates impregnated with AgNPs. The zones of inhibition show the maximum activity toward the test sample. In addition, crude extracts showed better inhibition when associated with AgNO3 and AgNPs. The data obtained from previous work showed similar results, which support the antibacterial activity of AgNPs. Less inhibition zone was seen in the controls (Figure 3).

Antimicrobial activity

In the present study, cytotoxicity of the methanolic crude extract in vitro and AgNPs was evaluated against 3T3 diabetic cell line at different concentrations. The samples indicated prominent cytotoxicity activity against the 3T3 cells. The data displayed that cell proliferation of 3T3 was inhibited significantly by methanolic crude extract: AgNPs with an IC50 value of 41.55 (μg/ml) of the crude extract and 47.19 (μg/ml) of the NPs. The percentage of toxicity increases with the concentration of AgNPs, proposing that synthesised AgNPs are important in medicine as antidiabetic agents. The percentage viability of the diabetic cells reduced with increased concentration of the samples, while cytotoxicity against 3T3 cell lines increased with the increased concentration of the samples (Figure 4). MTT assay of methanolic crude extract and AgNPs was performed. Of these, AgNPs showed better cytotoxicity results (Figure 5).

Antidiabetic activity

MTT Assay (3T3 cell line)

Comparative study of AgNPs and methanolic crude extract

DISCUSSION

Traditional medicine worldwide is redefined by research activities on various plants and their therapeutic values. Currently an epidemic expansion of DM has been reported worldwide. There are several therapeutic benefits of the different parts of the M. paradisiaca. However, most of the pharmacological properties of M. paradisiaca are based on anecdotal data and hence the present study was made to scientifically evaluate the antidiabetic property of M. paradisiaca tepal extract. Preliminary research showed the nontoxicity of the tepal extract on 3T3 cell lines.

The medicinal properties of the plant depend on the bioactive compounds that produce a certain physiological reaction on the human physiological process. Recently, some work has been done on phytochemistry, specifically on the banana flower of the Musa species. Preliminary screening phytochemical analysis of the air dried leaves and fruit peels from M. paradisiaca exposed the existence of glycosides, anthocyanin, tannins, flavonoids, and carbohydrate.6 A quantitative study on saponin and flavonoid was reported by Basha,21 and later, the phenolic content by Mazumder.22 The bark of M. paradisiaca was testified to comprise anthocyanins such as delphinidin, pelargonidin, peonidin, and malvidin.23,24 The total phenolic content in bracts was reported to be the lowest compared with that in other plant parts such as the rhizome and fruit peels. Some bracts are almost bioactive compounds whose structure is identical to insulin and function as ‘insulin-like elements,’ aiding in the treatment of type I and type II diabetes.

Almost all plants with antidiabetic activity showed to have secondary metabolites like glycosides, alkaloids and flavonoids.25 It has also been reported that numerous plants display effective antioxidant activity due to their phenolic compounds. Flavonoids and tannins are the phenolic compounds and plant phenolics are the main category of compounds that are primary antioxidant-free radical scavengers.26 Various in-vitro studies have shown that certain flavonoids are potent inhibitors of the oxidative modification of LDL by macrophages.27

Flavonoids play a crucial part in the treatment of diabetes28 as they can protect against hyperglycemic and alloxan-induced oxidative stress in experimental in vivo models.29 Plant alkaloids have the tendency to release insulin from pancreatic beta cells and also have the possibility to protect it from alloxan-induced pancreatic damage in experimental animals.30 Terpenoids as vitamins act as metabolism regulators and are protective antioxidants.31 The results of the present study indicate that the tepal extract contains biologically active ingredients of known pharmacological actions.

Antidiabetic potential and high synergistic antibacterial activity of silver nanoparticles synthesised

Nanotechnology includes research and technology development at the atomic, molecular or macromolecular levels. The methanolic extract was used to synthesise silver nanoparticles and showed that the silver nanoparticles was successfully synthesised and identified by the colour change of the extract. The characterised silver nanoparticles were analysed by the displayed SEM images. So far many studies have investigated the antioxidant and antibacterial features of M. paradisiaca.32 A comparative study was also done between the methanolic crude extract and nanoparticles. The results also showed that the Silver nanoparticles have more stable and better zone of inhibition against bacteria. Therefore the silver nanoparticles have many applications such antibioticidal, antioxidant properties. Antidiabetic activity of M. Paradisiaca against 3T3 cell line was confirmed against methanolic crude extract and silver nanoparticles. From the results it showed that the percentage of cell viability decreases and for cytotoxicity it increases. Hence it proves that M. Paradisiaca AgNPs has better antidiabetic activity.
Fig. 1: Comparative SEM image of Tepal Extract and Silver Nanoparticles at 10,000X.

Fig. 2: Antioxidant activity in different concentrations.

Fig. 3: Antimicrobial activity of crude extract and Silver nanoparticles against A) Escherichia coli, B) Pseudomonas aeruginosa, C) Staphylococcus aureus and D) Candida albicans.
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CONCLUSION

*M. paradisiaca* has been studied and the results of the present research findings show that the tepal extract was found to contain high phytochemical activity. Hence we can conclude that these plants are highly nutritious in nature. Phytochemical analysis concludes that it is rich in flavonoids, phenolic compounds, tannins, terpenoids, and phytosterol. In the present study, AgNPs showed the antioxidant activity of *M. paradisiaca*. The results showed that the methanolic extracts of these tepals also exhibit antioxidant potential and they are sources of natural antioxidant compounds. Comparatively, AgNPs showed the best antioxidant activity. This study aimed to find the link between ethnopharmacological claims and bioactive components in *M. paradisiaca* against all possible targets for cervical cancer through in vivo studies and molecular docking to develop a pharmacophore model for the active target. The biosynthesized AgNPs are predominantly spherical and nanosized. It is also found that tepal-mediated AgNPs exhibit excellent antimicrobial efficacy against tested human pathogens. This green method can be used as a better alternative source than chemical fabrication of nanomaterials and the biosynthesized NPs can be used in antibacterial medicines. The methanolic tepal extract of *M. paradisiaca* of AgNPs exhibited efficient antidiabetic activity in the diabetic rat model, so they may have potential for developing medical use.

REFERENCES


