Asian Journal of Pharmaceutical and Clinical Research

Online - 2455-3891
Print - 0974-2441

Vol 15, Issue 6, 2022

Research Article

ANTI-DIABETIC EFFECT OF GREEN SYNTHESISED SILVER NANOPARTICLES OF *PIPER BETLE*

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Received: 17 March 2022, Revised and Accepted: 21 April 2022

ABSTRACT

Objective: The objective of the current study is toward the green synthesis of silver nanoparticles from the aqueous leaf extracts of *Piper betle* (BL) and to evaluate its anti-diabetic efficacy.

Methods: Silver nanoparticles were prepared with the aqueous leaf extract of *Piper betle*. Characterization of silver nanoparticles prepared from extracts of *Piper betle* was done by UV-Visible spectrophotometer absorbance spectrum; Scanning Electron Microscope; and Fourier transform infrared spectrophotometer. The anti-diabetic efficacy was ascertained by inhibition assay of α-amylase activity and glucose diffusion inhibitory study with aqueous leaf extract of *Piper betle* and silver nanoparticles of *Piper betle* (BLNP).

Results: Based on the findings of spectrophotometer studies, it was confirmed that the silver nanoparticles were generated. High-density silver nanoparticles were synthesized by *Piper betle* was depicted by scanning electron microscope. The plant extracts (BLE) and its nanoformulation (BLNP) showed a constructive impact on controlling the glucose level by the inhibition of α-amylase activity and glucose diffusion.

Conclusion: It was concluded that the *Piper betle* can be employed as alternative medicine in the efficient management of Diabetes Mellitus.

Keywords: *Piper betle*, UV-visible spectrophotometer, Fourier transform infrared spectrophotometer, Nano formulation, α-Amylase activity, Glucose diffusion.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i6.44683 Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Urbanization has a greater impact on the day to activities of individuals, especially concerning diet patterns. The changes in lifestyle can affect the well-being of the biological system. One such metabolic disorder caused by dysfunction of insulin is hyperglycemia resulting in diabetes mellitus (DM) [1]. This elevated blood sugar ends up in long-term damage, dysfunction, and failure of vital organs. However, several hypoglycemic agents employed for the treatment of diabetes are reported to pose side effects, including liver dysfunction [2]. The present medication with synthetic drugs focuses on either augmenting the secretion and action of insulin or retard the glucose assimilation from the gastrointestinal tract. Synthetic drugs might pose deleterious side effects and therefore therapeutic intervention is aimed at herbal formulations in the management of diverse disorders. Globally, around 400 plants have been documented to possess anti-diabetic activity for which proper scientific validation is required substantiating their ability to maintain glucose homeostasis [3]. Phytochemical constituents such as saponins, phenols, and flavonoids studied in various plants such as *Proteus vulgaris*, *Euphorbia hirta*, and *Cassia glauca* showed potential α-amylase inhibitors. Pomegranate, *punica granatum* (L.) is an ancient fruit, this juice contains alkaloids and ellagic acid has been used as a remedy for diabetes in the Unani medicine practiced in the Middle East and India [4]. An earlier study with *Piper betle* leaf extract has documented the antioxidant [5] and antimicrobial [6] potency of the flora.

Researchers in the field of nanotechnology are gaining new insights into its versatile application and treatment of various diseases, including diabetes mellitus. Nanotechnology manipulates the size of the phytochemicals to the microscopic level to increase their bio-availability and exert their biological effect [7]. Research with organic extracts of plant material is widespread. Green synthesis of silver nanoparticles employs the production of AgNPs, from aqueous leaf extracts. The characterization of the formed AgNPs is essential before testing their biological potency. FTIR spectroscopy is an excellent tool analytical tool where the composition and structure of a functional group of the molecule can be determined by analyzing the position, width and intensity of acquired spectra [8]. The spectrum is characteristic of the organic molecules, which absorb infrared energy at specific frequencies so that the basic structure of compounds can be determined by the spectral locations of their IR absorptions [9].

The present study was designed to employ *Piper betle* (BL) leaves to decipher their impact on modulating the level of glucose. The usage of extracts in the native form poses reduced bioavailability which could be overcome by the green synthesis of silver nanoparticles of aqueous extracts of these leaves. These nanoparticles enhance the bioavailability of phytonutrients toward glucose homeostasis. Inhibition of α-amylase activity, the key enzymes responsible for hydrolyzing α,1-4 link in carbohydrate and inhibition of glucose diffusion were the basic parameters chosen to assess the impact of aqueous extract and their nano formulation in reducing the glucose load.

METHODS

Collection of plant material

The leaves of *Piper betle* were collected from the herbal garden at my residence and were authenticated by Prof. Dr. Chitti Babu, Head and Associate Professor, Department of Botany, Presidency College, Chennai - 600 005.

Preparation of aqueous extract of leaf

About 20 g of the BL leaves were thoroughly washed and were finely cut into small pieces. To this 100 ml of double distilled water was added and homogenized for 10 min. Further the extract was filtered with Whatman No.1 filter paper, stored at 4°C and used for further experiments [10].

Characterization of nanoparticles

UV-VIS absorption spectra

The UV-VIS absorption spectra of the synthesized silver nanoparticles using BL extract were measured using UV-visible spectrometer (Shimadzu UV- 2700).

Fourier transform infrared spectroscopy (FTIR)

The green synthesis silver nanoparticles of BLE were analyzed by FTIR. The pellets were prepared on a potassium bromide press. The spectra were scanned over the wave number range between 4000 and 400 cm^{-1} using IR Affinity-1s (Shimadzu, Japan) instrument.

Scanning electron microscopy (SEM)

The green synthesis silver nanoparticles from BL were loaded to carbon strips and sputter coated with gold. The sputtered samples were examined under SEM**.**

Inhibition assay for α -amylase activity

Five concentrations (25 μg/ml, 50 μg/ml, 75 μg/ml, 100 μg/ml, and 125 μg/ml) of BL plant extract and a positive control (acrabose) were prepared by dissolving in double distilled water. 100 μl of plant extract and 200 μl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5 mg/ml) were pre-incubated for 20 min at 37°C. To this 100 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5 s intervals. This reaction mixture was then incubated for 10 min at 37°C. The reaction was arrested by adding 200 μl of DNSA reagent. These test tubes were kept in a boiling water bath for 5 min and cooled to room temperature. Finally, this reaction mixture was diluted by adding 2.2 ml distilled water. The color intensity was measured at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 μl in distilled water. Control, representing 100% enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol and compared with the standard drug acarbose [11].

Glucose diffusion inhibitory study

1 ml of the BL aqueous leaf extract and its silver nanoparticles (BLNP) were placed separately in a dialysis membrane (12000 MW, Hi Media Laboratories, Mumbai, India) along with 22 mM glucose and 0.15 M sodium chloride. 1 ml of distilled water along with 22 mM glucose and 0.15 M sodium chloride placed separately in a dialysis membrane served as control. It was then tied at both ends using thread and it was immersed in a beaker containing 40 ml of 0.15 M sodium chloride and 10 ml of distilled water. The beakers were then placed on orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored every half hour. Three replications of this were done for 3 h [12].

Statistical analysis

Results will be expressed as mean ± standard error (S.E.) for groups of three observations. Differences were considered significant at $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS AND DISCUSSION

The folklore medicinal plants of Indian origin are our treasure as it is brimming with bioactive compounds, can be unfurled and used as alternative medicine. In the present study, *Piper betle* leaves were chosen to decipher their impact on modulating the level of glucose by studies on the inhibition of α-amylase activity and glucose diffusion. Research with organic extracts of plant material is widespread. Aqueous leaf extracts were employed for the present study to infer a simple way of acquiring phyto nutrients to manage DM. The application of plant extracts in the native form is restricted due to its decreased solubility and reduced bioavailability. These hindrances can be overcome by capping them on heavy metal like silver resulting in the production of nanocomposite. This paves way for the implication of nanotechnology in nutraceutical and therapeutics [13]. The formed nanoparticle has to be characterized before ascertaining it use for therapeutic interventions.

Characterization of nanoparticle

UV-VIS absorbance study of silver nanoparticle

In the present study, BLE exposed to silver nitrate showed a prominent absorption around 440 nm in contrast to BL extract with no such response (Fig. 1). This is supported by the color change of the extract to brown (Fig. 2). The color change of the extracts is due to the excitation of surface plasmon vibrations in silver nanoparticles [14]. The parameters like concentration of silver nitrate, the concentration of leaf extract and the duration may influence the formation of silver nanoparticles, hence characterisation of the silver nanoparticle is required. The absorption maxima of BLNP at 440 nm agree with the findings of *A. indica* leaf extract added to silver nitrate solution showing SPR at 436–446 nm [15]. The absorption maxima of the BLE plant extract at 440 nm due to the reduction of $AgNO_3$ ions in solution by the bioactive components is supported by the studies carried out with cannonball leaf extract [16].

Fourier transform infrared spectroscopy (FTIR) analysis of silver nanoparticle

The FTIR spectral study of silver nanoparticle is employed to ascertain the crucial role of the plant extract as a reducing and capping agent and confirm the functional groups available FTIR spectrum of green synthesized BLNP revealed clear peaks throughout the whole range of observation. A broad band at 3333.50 cm^{-1} represents O-H stretching and N-H stretching, intra-molecular hydrogen bonds between water and

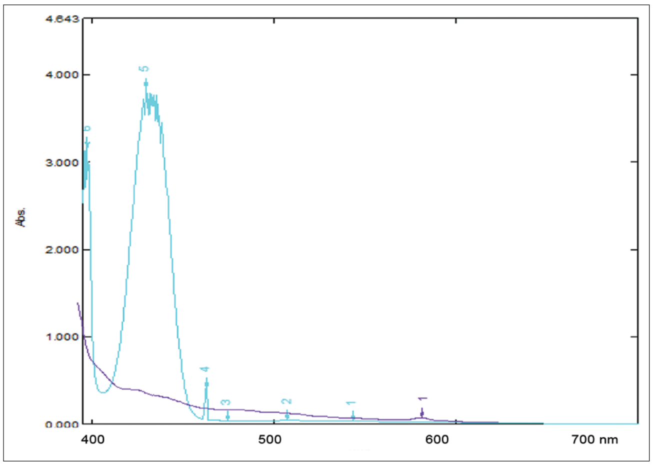

Fig. 1: Absorbance spectra of BLE and BLNP

Fig. 2: Synthesis of silver nanoparticles

bioactive components of plant material. The band at 2967.70 cm^{-1} can be assigned to C-H symmetric stretching vibration. The band at ~ 1650 cm^{-1} represents the C=O stretching of amide I revealing the alkyne group present in phyto nutrient of the extract. The band at 1067.10 cm^{-1} and 1100.40 cm^{-1} depicts asymmetric stretching of the C-O-C Bridge. The sharp peak with maximum intensity at ~ 500 cm^{-1} represents C-H out of plane bending vibration substituted ethylene systems –CH=CH (cis). The observed peaks are mainly attributed to flavonoids and terpenoids excessively present in plants extract (Figs. 3 and 4) [17] [18]. The peak at ~ 1800cm^{-1} reveals C - O stretching in esters and presence of C=O is depicted by the peak at ~ 1700cm^{-1} [19]. Band at ~ 1400cm^{-1} represents CH2 bending and CH3 symmetrical deformations. Form the FTIR spectral analysis, we infer that the bioactive components in the plant extract might play an important role in the synthesis and stabilization of silver nanoparticles by forming a strong coating on the nanoparticle. The spectral characteristics of FT – IR are shown in Table 1.

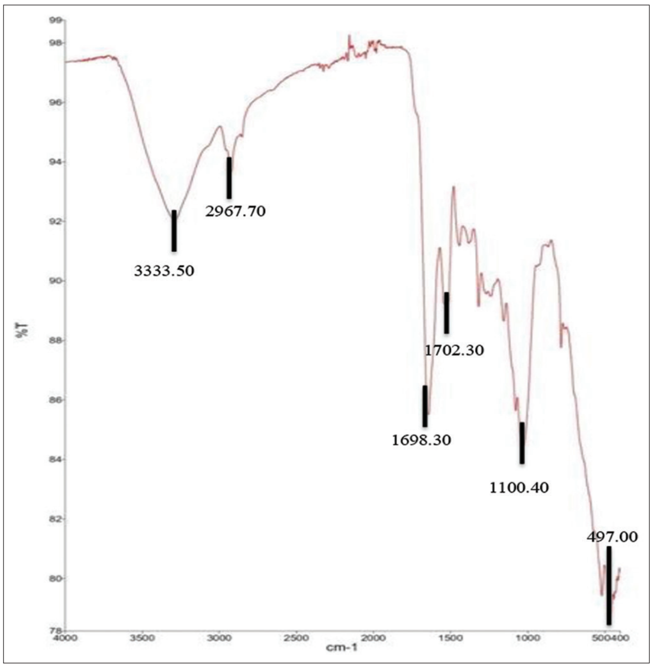

Fig. 3: FT-IR spectrum of BLE

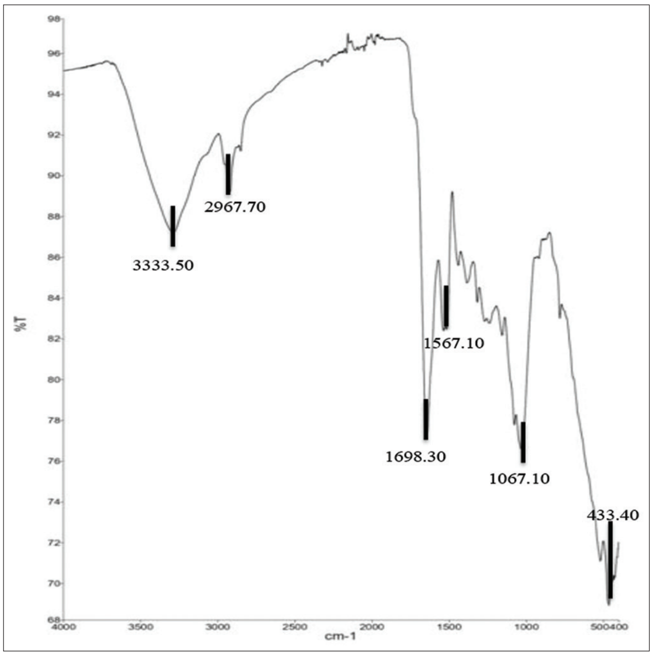

Fig. 4: FT-IR spectrum of BLE BLNP

Scanning electron microscopy (SEM)

Scanning electron microscopic (SEM) study high-density AgNPs synthesized by *Piper betle* leaf extract with the distribution of spherical and uniform BLNP with a diameter ranging between 60 nm and 100 nm is highlighted in Fig. 5. The average diameter of the NPs ranges from 1 μm to 20 μm [20].

In vitro studies

Traditional use of plants formulations serves as an excellent source of various therapeutic agents. Therapeutic interventions are focused at either triggering the endogenous insulin secretion and augment its action or impede glucose absorption [21]. The entry of glucose into the blood stream can be regulated by restricting the breakdown of carbohydrates through enzyme inhibition and the transport of glucose across the epithelial cells of the intestinal villi.

Inhibition assay for α -amylase activity

The *in vitro* α–amylase inhibitory activities of the green synthesized silver nanoparticles of BL were investigated. The result of experiment depicted that aqueous extract of BLE and its nanocomposite (25–125 μg/ml) exhibited a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The BLE showed inhibitory effect from 33% to 83% with an IC50 value of 50 μg/ml. The inhibitory effect in the range of 33–91% with an IC50 value of 50 μg/ml was recorded for BLNP. Acarbose is a standard drug for α-amylase inhibitor demonstrating α-amylase inhibitory activity from 33% to 66% with an IC50 value of 75 μg/ml. A comparison of α-amylase

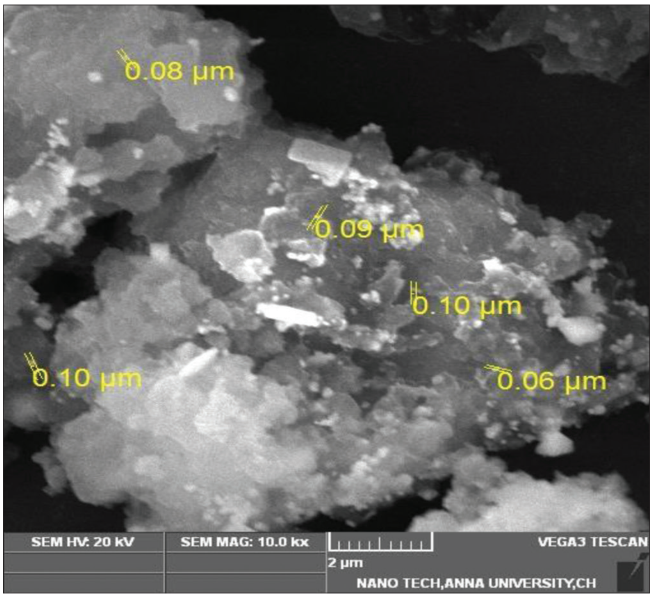

Fig. 5: SEM Image of BLNP

Table 1: Spectral characteristics of FTIR

Frequency	Assignment
~3300	O-H stretching and N-H stretching, intra-molecular hydrogen bonds
~2900	C-H symmetric stretching vibration
~1700	Presence of C=O
~1650	C=O stretching of amide I
~1100	asymmetric stretching of the C-O-C bridge
~500	C-H out of plane bending vibration substituted ethylene systems – CH=CH (cis)

inhibitory activity between the standard drug, plant extracts and nano formulation is been depicted in Fig. 6. The plant extract BLE showed maximum inhibition of α-amylase activity when compared BLNP. Hence, they can be employed as effective starch blockers since it reduces the availability of glucose in the body by impeding starch digestion mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose, and other simple sugars.

Glucose diffusion inhibitory study

The present study inferred a significant decrease in the diffusion of glucose to the external medium across the dialyzing membrane after 150 min when compared to the control (Fig. 7). This can be correlated to the time taken to complete the digestion and absorption of carbohydrates. Both in normal glucose tolerance test (GTT) and extended GTT, it was ascertained the absorptive capacity and the tolerance limit of the subject in effectively managing the glucose load. After 120 min, we expect complete absorption of the glucose ingested. In the present study, the diffusion of glucose is inhibited after 150 min and hence the plant extracts and the nanocomposites can be effectively employed in the management of DM. BLE which showed maximum inhibition of α-amylase activity did not have an equal effect in impeding the diffusion of glucose. Management of DM can de-scale the complications like hyperlipidemia and its associated hypertension [22]. Both the plant extracts and nano formulation (BLE and BLNP) showed a constructive impact on controlling the glucose level by the inhibition of α-amylase activity and glucose diffusion. It was concluded that plant preparations

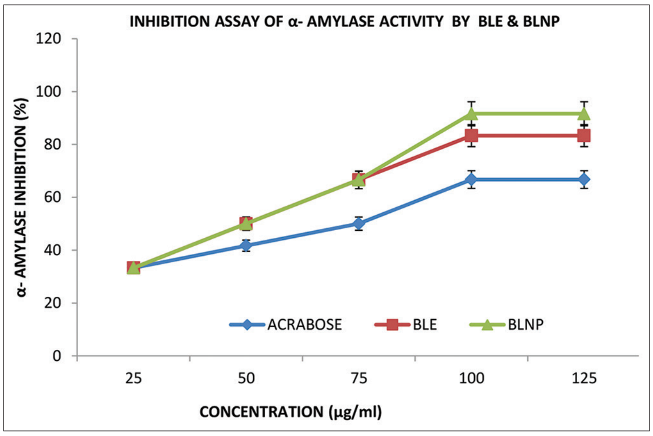

Fig. 6: Effect of aqueous extracts of BLE and its silver nanoparticles BLNP at varying concentration on α-amylase activity as compared to control. The figures are in percentage with the control showing 0% inhibition. The errors in measurement are indicated by the vertical bars

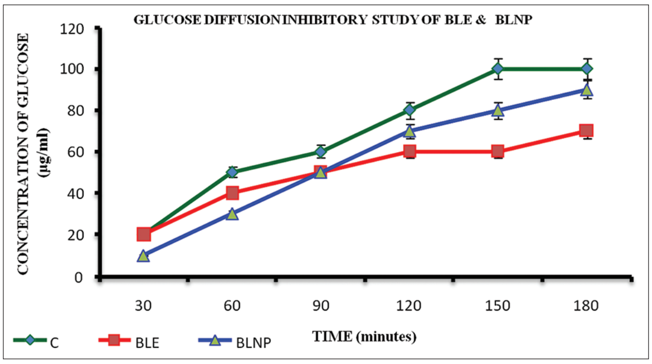

Fig. 7: Effect of aqueous extracts of BLE and its silver nanoparticles BLNP on the diffusion of glucose out of a dialysis membrane as compared to aqueous control. Values are mean ± standard error (S.E.) for groups of three observations with their standard errors indicated by vertical bars

taken regularly can have a positive effect in the management of DM and so can avoid secondary complications.

CONCLUSION

Native plants are well known by herbal pharmacologists for their medicinal properties. They are widely employed in Ayurveda and siddha formulations. This study provides a scientific evidence of their anti-diabetic effect. These plants are very affordable to the common man and can be easily incorporated in their daily diets. The restrictions posed on the use of the plant material in their native form can be overcome by capping them on to a heavy metal that also has antioxidant properties. This study can be further extended to design and develop anti-diabetic drugs free from harmful side effects. Thus these leaves can be used as an alternative medicine by humans especially the low socio economic community to manage diabetes, the lifestyle disorder by simple mastication of these leaves. Thus phyto-nanotherapy could create a prototype for further clinical trials in the management of other disorders.

ACKNOWLEDGMENT

The authors like to express their thanks to TAMIL NADU STATE COUNCIL FOR HIGHER EDUCATION (TANSCHE) for funding this project. They extend their heartfelt thanks to Centre for Nanoscience and Technology, Anna University, Chennai for the SEM Analysis. We extend our gratitude to Central Instrumentation Laboratory, Vels Institute of Science, Technology and Advanced Studies (Vistas), Pallavaram, Chennai, for timely help in FTIR Analysis.

AUTHORS CONTRIBUTION

Dr. N. Meenakshi: did concept, experiment design and planning the protocol. R. Angayarkanni: drafted manuscript. Dr. N. Meenakshi and R. Angayarkanni: Manuscript editing and review. A. Archana: Data analysis and interpretation.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

SOURCE OF FUNDING

The study was funded by TAMIL NADU STATE COUNCIL FOR HIGHER EDUCATION (TANSCHE).

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