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Comparative *in vitro* α-amylase Inhibitory Activity of *Carica papaya* Leaf Extract and Biosynthesised Gold Nanoparticles

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Abstract

The postprandial hyperglycemia that occurs in diabetes mellitus could be prevented or managed by the inhibition of the activity of pancreatic α-amylase. This study was carried out to comparatively check the inhibition potential of *Carica papaya* leaf extract and its biosynthesised gold nanoparticles (AuNPs). In this study, *Carica papaya* leaves were collected, and an extract was prepared; then, phytochemical screening of *Carica papaya leaves* was carried out. Further, this extract was used for the synthesis of AuNPs. Synthesised nanoparticles were characterised by FTIR, SEM, EDAX, and DLS for the analysis of surface functional groups, the size, elemental composition, zeta potential, and zeta size, respectively. Further standard methods for enzyme inhibition assay were approached to evaluate the anti-α- amylase activity of *Carica papaya* leaves and AuNPs. *Carica papaya* leaf (10-50mg/mL) and AuNPs (0.0625-1mg/mL) showed inhibition against the pancreatic α-amylase. The highest inhibition at 50mg/mL concentration of leaf extract exhibited 73% inhibition, and AuNPs exhibited 37.8% at 1mg/mL. The characterisation of AuNPs confirmed the functional groups, particle size, and elemental composition. These findings indicate that the crude leaf extract exhibited greater inhibition against pancreatic α- amylase than the biosynthesised gold nanoparticles. However, the study is limited to enzyme-based assays, and further biological validation is required to establish physiological relevance.

Major Findings: The leaf extract exhibited higher inhibition compared to AuNPs. Although AuNPs showed activity at lower concentrations, they did not achieve 50% inhibition within the tested range. These findings indicate that the crude extract demonstrated greater inhibitory effectiveness under *in vitro* conditions.

Keywords: Anti-α-Amylase Inhibition, Biosynthesised Gold Nanoparticles, *Carica papaya*, Pancreatic α-Amylase

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1. Introduction

Diabetes mellitus is a group of metabolic disorders described and recognised by the presence of hyperglycemia if not treated. Diabetes occurs due to errors in the secretion and action of insulin, and changes in the process of metabolism of carbohydrates, fat, and protein. The long-term complications occurring due to diabetes are retinopathy, nephropathy, and neuropathy[1]. Diabetes is detected worldwide in all populations and regions, such as rural and middle-income countries. Diabetes patients are increasing steadily, and an estimation according to the World Health Organisation, there were 422 million diabetic adults worldwide in the year 2014[2]; 578 million by the year 2030 and 700 million by the year 2045 [3]. An enzyme that essentially breaks down complex carbohydrates or starch in the digestive region is known as amylase, and this starch gets converted into simpler carbohydrates that as maltose, which is easily absorbed into the bloodstream by the intestine. The pancreatic amylase and salivary amylase are the abundant isoforms of amylase, and the pancreas produces the pancreatic amylase, which is manufactured

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by acinar cells and secreted into the gastrointestinal tract[4]. A therapeutic way for diabetes treatment or management could be the reduction of hyperglycemia by inhibiting carbohydrate digestion in the intestine, which is accompanied by pancreatic α-amylase[5]. Inhibition of this pancreatic α--amylase that digests carbohydrates into smaller units could significantly decrease the blood glucose level post-meal, as starch digestion gets delayed[6]. A- amylase inhibitors that are currently available in the market are acarbose, miglitol, metformin, etc., and are synthetic hypoglycemic and non-specific agents that have various side effects[5]. Therefore, natural extracts can be better and safer agents for the treatment of diabetes, as various medicinal plants can be studied as antidiabetic agents. There are around 800 medicinal plants according to the *Ayurveda* in the Indian subcontinent, which have antidiabetic properties[7]. Gold nanoparticles possess advanced features associated with diagnostics, therapeutics, and molecular nanoprobes, and gold also has great significance in the synthesis of nanoparticles because of its electronic and optical properties based on size. Gold nanoparticles are found to be biocompatible, inert, easily bind with a wide range of biomolecules, and give a large surface area for the immobilisation of these biomolecules[8]. Here, in the research work, *Carica papaya* is selected based on its properties. It is a plant with medicinal properties which usually scattered in the tropical areas of Africa, a native of Mexico and northern South America, now naturalised in many parts of the world, including tropical and subtropical regions. *Carica papaya* plant parts, except fruit, as waste materials, consolidate limitless possibilities for new medications[9]. Therapeutic applications of *Carica papaya* concerning the antioxidant activity associated with tremendous necessary micronutrients and several secondary metabolites found in the *Carica papaya* seeds, with negligible toxicity[10]. Leaves of *Carica papaya* possess various beneficial properties. It is known to treat dengue fever; papaya leaf extract enhances the number of white blood cells and platelets, which helps in clotting and liver repair. The leaf extract of *Carica papaya* inhibits cancer cell growth and shows enhanced production of Th1-type cytokines (key signalling molecules), which are known to regulate immunity. Also, this extract is known to be used as medicine in the treatment of malaria. The leaves are also known to facilitate digestion as they contain the enzyme carpain, which acts as bactericidal and kills microbes that hinder the digestion process. Other medicinal uses of *Carica papaya* Leaves are such as in acne treatment, meat tenderiser, boosting appetite, relieve menstrual pain and nausea[11,12]. Here, in the research study, the inhibition of α-amylase activity of *Carica papaya* leaves was explored, and the mode of inhibition was evaluated. The gold nanoparticles were synthesised using *Carica papaya* leaf extracts, and their inhibitory effect was determined against pancreatic α-amylase. Recent studies have demonstrated that plant-derived phytochemicals exhibit significant α-amylase inhibitory activity due to the presence of phenolic and flavonoid compounds[13]. Additionally, green synthesis of gold nanoparticles using plant extracts such as *Carica papaya* has gained considerable attention due to its eco-friendly and sustainable approach[14,15]. Biosynthesised nanoparticles have also been investigated for their ability to modulate enzyme activity, including α- amylase inhibition, under *in vitro* conditions[16]. However, limited studies have comparatively evaluated the inhibitory potential of crude plant extracts and their nanoparticle formulations. Therefore, the present study aims to investigate whether biosynthesised gold nanoparticles alter the α- amylase inhibitory activity of *Carica papaya* leaf extract.

2. Materials and Methods

2.1 Materials and Reagents

Porcine pancreatic α--amylase (PPA) (Cas No. 9000-90-2) purchased from Sisco Research Laboratories (SRL), Tetra chloroauric acid ($HAuCl_4$), Starch, 3, 5 - dinitro salicylic acid (DNSA), Sodium phosphate buffer (0.02M, 6.9pH), Iodine reagent, chloroform, ferric chloride, ferrous chloride, lead acetate, hydrochloric acid (HCl), Sulfuric acid (H_2SO_4), Mayer's reagent (mercuric chloride and potassium iodide), Ehrlich reagent, aqueous and alcoholic sodium hydroxide glacial acetic acid, concentrated nitric acid and ammonia were obtained from Sigma Aldrich.

2.2 Collection of Plant Material

Carica papaya leaves were collected from the Charak Udyan, Jiwaji University, Gwalior, from January to October. The collected leaves were washed using tap water, dried at room temperature, converted into powdered form and stored until use.

2.3 Preparation of Extract

Aqueous extracts of *Carica papaya* leaves were prepared using leaf powder, and then the dried leaf powder was dissolved in double-distilled water. Secondly, the extract

was kept on the shaker for 2-3 hrs. after dissolution and then kept overnight at room temperature for better extraction. After 24 hrs., the extract was filtered using muslin cloth followed by Whatman paper, and the filtrate was dried at 25-30 ◦ C. The dried filtrate was stored and used for further tests[17].

2.4 Phytochemical Analysis

Biochemical test for the confirmation of Tannins was performed using the Ferric chloride test, and Mayer's test was performed for the confirmation of Alkaloids. The confirmation of Glycosides was done using the Keller-Killani test, the confirmation of Saponins through the foam test and flavonoids was confirmed using Shindo's test. Biochemical tests for the confirmation of phenols and Catechins were performed, and steroids were confirmed using Salkowski's test. The biochemical tests for the confirmation of Coumarins, Quinones, Xanthoproteins, Sterols and terpenoids were performed[18].

2.5 Biosynthesis of Gold Nanoparticles

The gold nanoparticles (AuNPs) were synthesised using *Carica papaya* leaf extract. 50 ml aqueous solution of 1mM $HAuCl_4$ was prepared, and 2 ml aqueous leaf extract was added to it dropwise and kept on a magnetic stirrer (27˚C, 250-400 rpm) for continuous stirring for the synthesis of nanoparticles. After the formation of suspension and colour change of the mixture from light yellow to reddish brown, the reaction mixture was kept for 2 hours on the magnetic stirrer. After 2 hours of stirring on a magnetic stirrer, the reaction mixture was kept for drying on the hot plate at 50-60 ˚C and then the synthesised gold nanoparticles were washed with 100% ethanol 3-5 times. After washing, the synthesised gold nanoparticles were kept in a dry bath for drying at 80˚C. The biosynthesised gold nanoparticles were further used for the evaluation of inhibitory activity against pancreatic α-amylase[19,20].

2.6 Characterisation of Biosynthesised Gold Nanoparticles

The following biophysical methods were used to characterise the biosynthesised gold nanoparticles.

2.7 FTIR Spectroscopy Analysis

In the FTIR analysis, the lyophilisation of the biosynthesised metal nanoparticles is carried out and combined with KBr pellets, and then subjected to a wide range of FTIR spectral analyses to obtain various peaks for the samples. The FTIR spectroscopy analysis leads to the determination of the surface functional group[21].

2.8 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to characterise the biosynthesised metal nanoparticles. The sample is placed as a single drop on the sample stage, and for 5 min, the aqueous solvent will be left to evaporate. The gold coating was made on the dried sample using an ion sputter-coater with a gold target, and then the size and shape at various magnifications will be analysed[22].

2.9 Energy Dispersive X-Ray Spectroscopy (EDX)

EDX is used for the determination of the elemental composition of the biosynthesised metal nanoparticles. The spectrometer uses the electron beam (10-20 keV) that strikes the surface of the sample, which leads to the emission of X-rays from the sample. The electron beam gone through the sample gives the elemental image of the sample[23].

2.10 Dynamic Light Scattering (DLS)

DLS is an analysis technique used to evaluate the charge on the surface of nanoparticles via zeta potential analysis and the average size of nanoparticles via zeta size analysis. Zeta potential is measured by adding the sample solution to the electrodes, and the potential is analysed by the Doppler method. Zeta Size and Zeta potential were analysed through Malvern Zetasizer[24].

2.11 Enzyme Inhibition Assay

2.11.1 Qualitative Assay – Starch Iodine Test

The qualitative enzyme inhibition assay was performed with different concentrations of leaf extract (10 mg/mL to 50 mg/mL) and AuNPs (0.0625 mg/mL to 1 mg/mL). Test tubes with 0.5 ml PPA solution and 0.5 ml test sample solution (aqueous leaf extract and biosynthesised gold nanoparticles) were kept for pre-incubation at 37˚C for 10 min. After 10 min pre-incubation, 0.5 ml of 1% starch solution was added to the test tubes and were kept for incubation at 37˚C for 15 minutes. After 15 min, a few drops of iodine reagent were added to all tubes for colour observation. The blue-black/red-brown colour was observed as a result of inhibition[25].

2.11.2 Quantitative Assay – DNSA Method

The quantitative enzyme inhibition assay was performed with different concentrations of leaf extract (10 mg/mL to 50 mg/mL), AuNPs, and acarbose (0.0625 mg/mL to 1 mg/mL). Test tubes containing 0.5 ml of PPA solution and 0.5 ml of test sample solution (aqueous leaf extract, biosynthesised gold nanoparticles, and acarbose) were pre-incubated at 37˚C for 10 min. After 10 minutes, 0.5 ml of 1% starch solution was added to all test tubes. All test tubes were kept for incubation at 37˚C for 15 minutes. After 15 minutes, 1 ml of DNSA reagent was added to all tubes and kept in a boiling water bath for 5 minutes. After 5 minutes, all test tubes were kept for cooling at room temperature and then diluted with distilled water. After dilution, the absorbance is noted for all reaction mixtures at 540nm[26]. The absorbance obtained from the quantitative enzyme inhibition assay was used for the determination of 50% inhibition concentration. The inhibition percentage was calculated from the following formula:

$$\text{Inhibition\%} = [O.D_{NC} - O.\ D_{test} / O.D_{NC}] * 100$$

where, $O.D_{NC}$ = Absorbance of negative control

$O.\ D_{test}$ = Absorbance of test sample

All experiments were performed in triplicate (n=3), and results are expressed as mean ± SD.

3. Results and Discussion

3.1 Phytochemical Analysis of *Carica papaya* Leaf Extract

The various phytochemicals were screened using an aqueous extract of *Carica papaya* leaves. The phytochemical tests for the confirmation of tannins, alkaloids, glycosides, flavonoids, phenols, quinones, saponins, steroids, coumarins, xanthoproteins, and sterols, and terpenoids showed positive results, whereas tests performed for the confirmation of catechins showed negative results. Thus, most of the phytochemicals were found in the aqueous leaf extract of *Carica papaya* (Table 1), making the extract to be used as natural inhibitor.

3.2 Biosynthesis of Gold Nanoparticles

The synthesised gold nanoparticles were confirmed primarily through visual observation, as after one hour of the addition of *Carica papaya* dropwise, the pale-yellow colour of gold metal salt changes to a reddish-brown colour, indicating the reduction of gold metal salt. The colour change of the solution also indicates the size and shape of the synthesised nanoparticle[21] (Figure 1).

Table 1. Results of phytochemical analysis (+ present, - absent)

Phytochemicals	Aq. leaf extract
Tannins	+
Alkaloids	+
Glycosides	+
Saponins	+
Flavonoids	+
Phenols	+
Steroids	+
Catechins	-
Coumarins	+
Quinones	+
Xanthoproteins	+
Sterols and Terpenoids	+

(+ present, - absent)

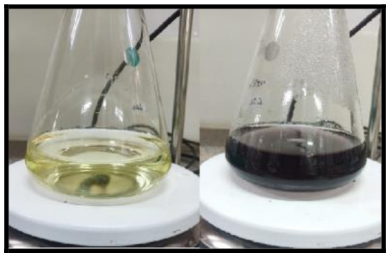


Figure 1. The synthesis of gold nanoparticles using the leaf extract of *Carica papaya.*

3.3 Characterisation of Biosynthesised Gold Nanoparticles

3.3.1 FTIR Spectroscopy Analysis

FTIR analysis is a characterisation method commonly used to identify functional groups of the phytochemicals present in the sample. The analysis is carried out with the help of peak values in the range 400-4000 cm^{-1} that tell about the bioactive compound in natural products (Selvamani, 2019). Here, in the study, the FTIR analysis was used to determine the functional groups of the present phytochemicals in the biosynthesised gold nanoparticles of the leaf extract of *Carica papaya*. The FTIR result showed various peaks at different frequencies [wavenumber (cm^{-1})] (Figure 2), indicating different

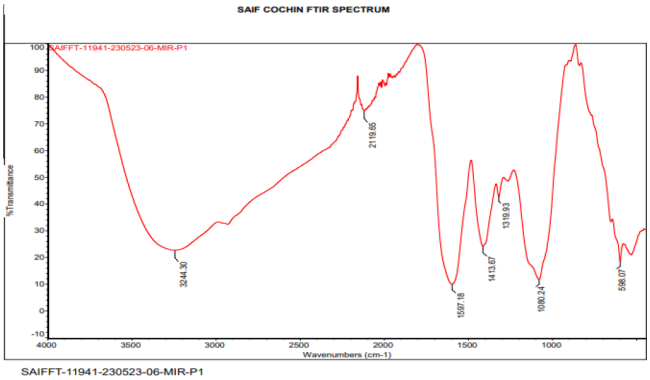


Figure 2. FTIR spectra of the biosynthesised gold nanoparticles.

Table 2. Various peaks from FTIR spectra and associated functional groups

Peak cm^{-1}	Functional groups
3244.3	O-H Stretch
2119.65	C≡C Terminal alkyne (monosubstituted)
1597.18	N-H bend
1413.67	C-C Stretch
1319.93	Di alkyl/aryl sulfones
1080.24	C-N Stretch
598.07	C-Br Stretch

functional groups. The analysis showed peaks (Table 2) at 3244.30 cm^{-1}, 2119.65 cm^{-1}, 1597.18 cm^{-1}, 1413.67 cm^{-1}, 1319.93 cm^{-1}, 1080.24 cm^{-1}, and 598.07 cm^{-1} [27,28]. The functional groups associated with the peaks revealed were due to the phytochemicals present in the leaf extract that were part of the reducing and capping agent during the synthesis of nanoparticles.

3.3.2 *Scanning Electron Microscopy (SEM)*

The SEM analysis is an analysis that tells about the surface morphology and size of the synthesised nanoparticles. The analysis was performed using the dried samples placed on glass slides, followed by a coating of a gold thin layer with the help of vacuum sputtering. After this, the nanoparticle was observed under scanning electron microscopy, and the image of the nanoparticle was obtained. The SEM analysis showed the surface morphology and size of the biosynthesised gold nanoparticles (Figure 3). The image shows some agglomeration of nanoparticles with the size of the nanoparticles under the range of 100nm.

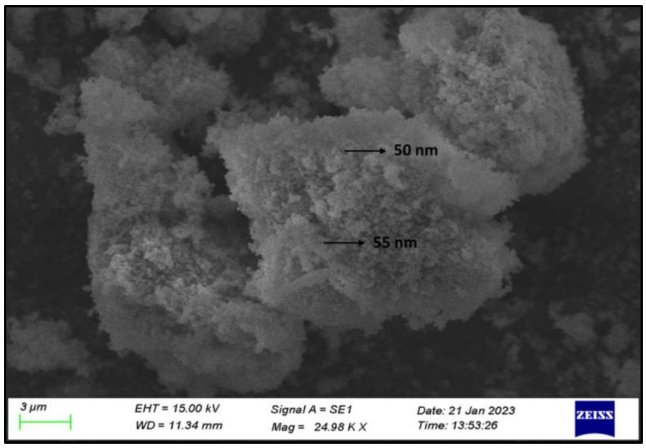


Figure 3. Scanning Electron Microscopy image of the biosynthesised gold nanoparticles.

3.3.3 *Energy Dispersive X-Ray Spectroscopy (EDX)*

Energy Dispersive X-ray analysis: an X-ray technique employed to evaluate the elemental composition of the sample. The spectra obtained through EDX show the percentages of the present elements in the sample. The EDX technique gives detailed information about the elemental composition that allows the identification of the presence and relative quantities of the elements in the sample[11,29]. The EDX analysis of the nanoparticles showed various peaks of different elements, as shown in Figure 4. The peaks show the elemental composition of the nanoparticles, depicting Carbon, Oxygen, Chlorine, and gold elements. Carbon and oxygen are present due to the plant extract used, and chlorine and gold are present due to the metal salt used ($HAuCl_4$)[30].

3.3.4 *Dynamic Light Scattering (DLS)*

The zeta potential of the biosynthesised gold nanoparticles was found to be -21.5 mV (Figure 5a). The value of zeta potential obtained near the +30mV/-30mV tells about the stability and instability of the nanoparticles. The values were more negative or more positive than -30mV and +30mV, respectively, showing that the nanoparticles synthesised are moderately stable[31]. The zeta average size of the nanoparticles was found to be 55.75 nm in diameter (Figure 5b). The size showed the formation of nanoparticles, as the size was under the range of 100nm, fulfilling the definition of nanoparticles[32].

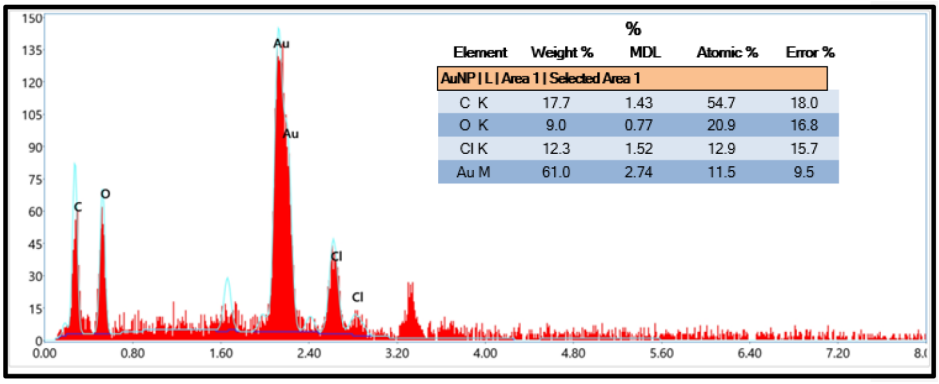
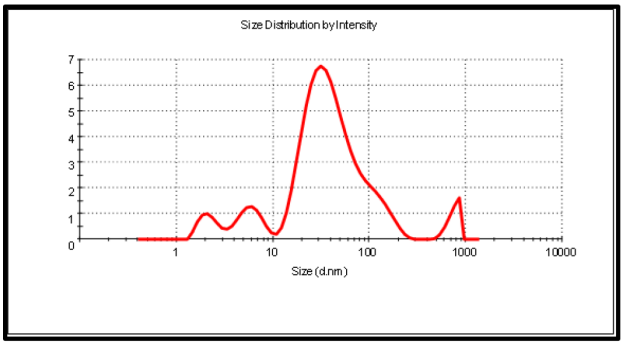
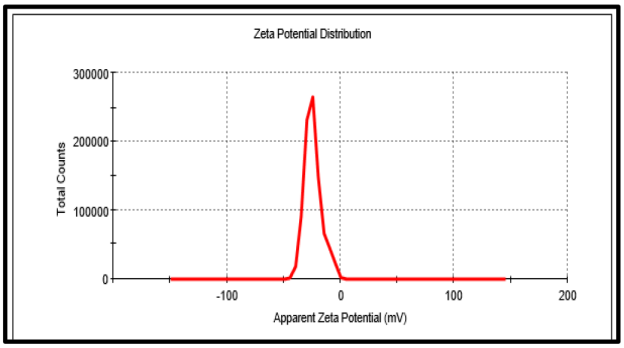


Figure 4. Energy dispersive X-ray spectroscopy spectrum and elemental composition.



(a)



(b)

Figure 5. **(a)** Zeta size and **(b)** zeta potential of the biosynthesised gold nanoparticles.

3.4 Enzyme Inhibition Assay

3.4.1 Qualitative Enzyme Inhibition Assay

The qualitative enzyme inhibition assay performed using the starch iodine test showed the colour difference with increasing concentration of leaf extract (10mg/mL to 50mg/mL), and AuNPs (0.0625 mg/mL to 1 mg/mL) didn't show a significant colour difference. Also, there was an observation that the colour of the plant extract hinders the colour of the starch iodine complex, due to which the analysis of enzyme inhibition based on visual observation becomes difficult[33,34]. The result obtained based on the colour indicated that the inhibition occurred at 40mg/mL and 50mg/mL concentration of leaf extract, and in the case of AuNPs, there was less inhibition as compared to the leaf extract.

3.4.2 Quantitative Enzyme Inhibition Assay

The quantitative enzyme inhibition assay performed using the DNSA method of leaf extract (10mg/mL to 50mg/mL), biosynthesised gold nanoparticle, and acarbose (0.0625 mg/mL to 1 mg/mL) showed inhibition with increasing concentration (Table 3). The enzyme inhibition percentage was calculated from the absorbance of the quantitative assay. The results obtained from the inhibition percentage indicated that the phytochemicals present in the leaf extract and biosynthesised gold nanoparticle containing the phytochemicals of the leaf extract, which enhanced the activity of the nanoparticle, played the role of inhibitor. The *Carica papaya* leaf extract and biosynthesised gold nanoparticle as α-amylase inhibitor inhibited the activity of porcine pancreatic α-amylase.

The present study demonstrated that *Carica papaya* leaf extract exhibited higher α-amylase inhibitory activity compared to biosynthesised gold nanoparticles. The higher inhibitory activity of the leaf extract may be attributed to the presence of bioactive phytochemicals such as flavonoids and phenolic compounds. These compounds are known to interact with enzymes and inhibit their activity[10,21]. The reduced activity observed in AuNPs suggests that nanoparticle formulation did not enhance enzyme inhibition under the present conditions. This may be due to alteration or reduced

Table 3. Inhibition percentage of different concentrations of leaf extract, AuNPs and acarbose

Concentration of leaf extracts (mg/mL)	Inhibition percentage (%)	Concentration of AuNP (mg/mL)	Inhibition percentage (%)	Concentration of acarbose (mg/mL)	Inhibition percentage (%)
10	16.6±4.5	0.0625	21±2	0.0625	72.67±0.05
20	45.16±6.7	0.125	22.12±1.2	0.125	88.96±0.04
30	63.44±2.5	0.25	26±2.2	0.25	96.93±0.02
40	59.25±2.9	0.5	30.8±1.15	0.5	97.26±0.03
50	73.11±2.3	1.0	37.8±3.1	1	99.89±0.01

Results are expressed as mean (n=3, ±SD)

availability of active phytochemicals upon nanoparticle synthesis. Similar findings have been reported where crude extract demonstrated stronger enzyme inhibition compared to nanoparticle formulations[12,22]. Although nanoparticles are often associated with improved bioavailability, such effects were not evident in this *in vitro* study. The study has several limitations. The DNSA method is susceptible to interference from reducing sugars and plant phenolics. The qualitative starch-iodine assay showed limited reliability due to colour interference[25]. Additionally, the study is limited to *in vitro* enzyme assays and lacks cellular or *in vivo* validation.

4. Conclusion

The findings of the research work showed the inhibition potential of *Carica papaya* leaf extract and biosynthesised gold nanoparticles from the leaf extract. The pancreatic α-amylase inhibition was evaluated using *Carica papaya* leaf extract and its biosynthesised gold nanoparticles across different concentrations. The obtained data were compared with the negative control (pure enzyme) and positive control (acarbose) for the analysis of the outcome. The leaf extract exhibited the highest inhibition (73% at 50mg/mL), whereas AuNPs showed 37.8% inhibition at 1mg/mL. Although AuNPs showed inhibitory activity at a lower concentration, they did not achieve 50% inhibition within the tested range, indicating comparatively lower effectiveness. These findings suggest that while nanoparticle formulation may influence activity at lower doses, the crude extract remains more effective under the *in vitro* conditions. The observed activity of AuNPs at lower concentrations may indicate potential dose-dependent advantages which require further investigations. Future research should focus on evaluating the biological activity using cellular and *in vivo* models, identifying active compounds responsible for inhibition, and optimising nanoparticle synthesis to enhance functional properties.

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Author's Contributions

Conceptualisation, S.V, S.T.R and N.P.; methodology, S.V and N.P.; validation, S.V, S.T.R and P.A.K.; formal analysis, N.P and S.V.; investigation, N.P and S.V.; resources, S.T.R and S.V.; data curation, S.V, S.T.R and P.A.K.; writing-original draft preparation, N.P and S.V.; writing-review and editing, N.P and S.V.; visualisation, N.P and S.V.; All authors have read and approved the published version of the manuscript.

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