Eco. Env. & Cons. 30 (May Suppl. Issue) : 2024; pp. (S441-S446)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i03s.077

α-amylase and α-glucosidase enzyme inhibition *In vitro*, antioxidant properties, and green synthesis of *Tabebuia rosea* DC-mediated FeNPs synthesis

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(Received 2 January, 2024; Accepted 7 March, 2024)

ABSTRACT

Tabebuia rosea DC, is a well-known plant used in traditional medicinal practices for several human ailments. The goals of the research included a phytochemical analysis and assessment of *Tabebuia rosea* DC or bark and flower extract's, Antioxidant, Antidiabetic effects. The enzyme inhibition tests for α-amylase and α-glucosidase were used for experimental purposes, anti-diabetic research, including antioxidant research. The plant *parts of* bark and flower extract which contains of flavonoids and polyphenols. These phytochemicals exhibited the very greatest antioxidant activity of at different concentrations level. *Tabebuia rosea* Dc displayed better antidiabetic conditioning with IC-50 maximum of 309.23 $\mu g/ml^{-1}$ and minimum of inhibition 261.76 $\mu g/ml^{-1}$. and inhibition of α-amylase activity were observed at 272.42 $\mu g/ml^{-1}$ mg/ml concentration for the protein extracts from bark FeNps and flower FeNps and acarbose, respectively. alpha glucosidase respectively and standard Acarbose respectively. The IC-50 were (bark and flower) 311.21, 281.82, $\mu g/ml^{-1}$ and 251.29 $\mu g/ml^{-1}$ for FeNPs extract standard Acarbose, respectively. The findings of this study emphasise the herb *Tabebuia rosea* DC is good cytotoxic, Antioxidant, and *in-vitro* Anti-diabetic properties.

Key words: *Tabebuia rosea DC Antioxidant activity, Alpha-amylase, Alpha-glucosidase*

Introduction

The green conflation of iron nanoparticles using colorful factory excerpts has been reported by numerous experimenters. The biosynthesis of iron nanoparticles (Fe NPs) has been substantially performed from of green tea which is a cheap and original resource (Saif *et al*., 2016). Iron nanoparticles (FeNps) are the smallest flyspeck of iron essence with a large face area and high reactivity. They are non-toxic FeNps have excellent dimensional stability, high thermal and electrical conductivity, high face area, and are largely glamorous. The nano particle of FeNps can oxidize incontinently when exposed to water or air and produces free Fe ions. There are multitudinous operations of FeNps but the most promising one includes their in medicine delivery (Batool *et al*., 2021).

Herbage conflation has numerous advantages compared to chemical and physical styles it is non-toxic (Ying *et al.,* 2022). The natural approach which includes different types has been used to synthesize different metallic NPs, which has advantages over other chemical styles as this is greener, energy- saving, and cost-effective. cost-effective natural motes on the face of NPs make them biocompatible in com-

parison with the NPs prepared by chemical styles (Ying *et al.,* 2022). Diabetes is one of the oldest conditions known to humanity and is also a leading cause of death in utmost developed, developing, and recently industrialized countries (Senhaji *et al*., 2022). Diabetes mellitus (DM) is a patient metabolic complaint attributed to hyperglycemia, and hyperactive amino acidemia, together with, insulin- receptor resistance.

Among the different types of DM, type 2 is lesser general worldwide (Jayappa *et al*., 2020)

A different range of insulin tributes and bioactive metabolites are discovered in the medicinal shops alienated with their factors. These restorative shops are rich in tannins, phenolics, and alkaloids. similar biologically active substances set up in shops may accelerate the process by which essence ions are converted into biologically active nanoparticles in a normal biogenesis pathway that's salutary to the terrain (A, 2023). Hyperglycemia in diabetes mellitus can produce bus- oxidation of glucose, protein glycation, and polyol pathway activation metabolism, therefore adding the conformation of reactive oxygen species (ROS) inordinate ROS product will lead to oxidative stress (Godavari and Amutha, 2017). These are also acted on by nascence glucosidases and further degraded to glucose that on immersion enters the bloodstream. Declination (Kajaria *et al*., 2013).

Plant collection and Authentication

The plants of shops were collected from the trees growing around the original areas of, Thanjavur. The factory were authenticated by a Taxonomist, and an exemplar instance were deposited at the Department of Botany, St. Joseph's College, Tiruchirappalli, India. The collected factory material were washed with double-disstilled water to exclude any face contaminations, and latterly diced into small fractions. The bark and flower were also subordinated to shade drying for a period of 7- 10 days (Vijayaraj and Sri Kumaran, 2018)

Preparation of Plant Extract

The bark and flower were uprooted to a fine grease-paint using a mixer grinder. An aggregate of 100 g of the grease paint were also dispersed in 100 ml of distilled water and boiled at 60 ^{o}C for 20 twinkles. After cooling, the excerpt were filtered using a Whatman No. 1 sludge paper and stored in a refrigerator for further disquisition (Hassan *et al*., 2022).

Green Synthesis of Ferrous Oxide Nanoparticles

The conflation of Ferrous oxide nanoparticles were carried out using bull ferrous and factory excerpt. A result of 0.1 M The conflation of Ferrous oxide nanoparticles were carried out using bull Ferrous and factory excerpt. A result of 0.1 M bull sulfate in double distillate sulfate were prepared. ferrous sulfate and factory excerpt were mixed in rates of 55, 64, 73, 82, and 91 within 1 hour, the admixture turned green in color. The entire response process was conducted in the dark. The performing suspense were also centrifuged at 15,000 rpm for 15 beats, and the bullet containing ferrous oxide nanoparticles were werehed 3-4 times with deionized water to remove contaminations. The rained nanoparticles were latterly lyophilized and stored in a cool, dry, and dark place for farther characterization (Manivannan *et al.,* 2023).

Antioxidant activity (DPPH free radical scavenging activity)

DPPH free radical scavenging activity

DPPH free radical scavenging activityof extracts were determined by the method of (Sobiyana *et al.*, 2019).

Total antioxidant activity

The Total antioxidant activity power of extracts were determined by the method of (Zhang *et al.*, 2014).

Superoxide scavenging activity

Evaluation of superoxide anion-scavenging activity of were determined by the method (Ogundajo *et al.*, 2018).

Nitric oxide scavenging exertion

Evaluation of nitric oxide scavenging activity of were determined by the method (Sobiyana *et al*., 2019).

Reducing power activity

The Fe^{3+} reducing the power of extracts were determined by the method of Oyaizu (Irshad *et al.,* 2012).

Methods

***In Vitro* Antidiabetic Activity**

In vitro α-amylase inhibition study

In vitro α-amylase inhibition assay were carried out by the method of Reagents (Apostolidis *et al*., 2007)

1. 20 mM Phosphate buffer (pH 6.g rea se paints (0.5 mg/ml)
2. 0.5% starch
3. 96 mM 3,5-dinitrosalicylic acid (DNS)

Procedure

Colorful attention of the sample were prepared 100 µg/ ml, 200 µg/ml, 300 µg/ ml, 400 µg/ ml, and 500 µg/ ml using phosphate buffer(pH6.9). 500 µl of sample and 500 µl of 20 further hate buffer pH 6.9, containing α- amylase at a attention of 0.5 mg/ml were incubated at 25 °C for 10 min. After pre-incubation, 1000 µl of 0.5 bounce result in 20mM phosphate buffer, pH 6.9, were added. The response fusions were also incubated at 25 °C for 10 min. The response were stopped with 500 µl of 96 mM 3, 5-dinitro salicylic acid (DNS) color reagent. The test tubes were also incubated in a boiling bath for 5 min and cooled to room temp. Absorbance (A) were measured at 540 nm. Acarbose were used as positive control and the inhibitory exertion of α- amylase and the percent of inhibition were calculated.

Control incubations represent 100 enzyme exertion and were way by replacing excerpts. For blank incubation (to allow for absorbance produced by the sample), enzyme result were replaced by buffer result and absorbance were recorded. Separate incubation carried out for response t = 0 were performed by adding samples the Deth NS result incontinently after the addition of the enzyme.

***In vitro* α-glucosidase inhibition study**

The α-glucosidase inhibitory activity were determined according to the method described by (Apostolidis *et al.*, 2007)

1. Yeast a-glucosidase were dissolved at a concentration of 0.1 U/ml in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 2000 mg/ml and sodium azide -200 mg/ml
2. Paranitrophenyl-α-D-glucopyranoside -5 mM

500 µl of the Difference attention (100 µg/ ml, 200 µg/ ml, 300 µg/ ml, 400 µg/ ml, and 500 µg/ ml) of sample and 100 µl of incentive α- glucosidase result were incubated at 25 excerpts 10 min followed by the addition of 50µl of Paranitrophenyl- α- D-glucopyranoside result in 0.1 spook l- 1 phosphatebuffer (pH 6.9) of 0.1 replying admixture were also incubated at 25 °C for 5 min and the absorbance were read at 405 nm. Acarbose were used in a positive control and the inhibitory exertion of glucosidase- were calculated.

Control incubations represent 100 enzyme exertion and were conducted also by replacing excerpts. For a blank incubation (to allow for absorbance produced by the sample), enzyme result were replaced by a buffer result, and absorbance were recorded.

Statistical analysis

Tests were carried out in triplet for 3 separate trials. The results were graphically determined by a direct retrogression system using Window- grounded Padedpad Instat (interpretation 3) further. Results were expressed graphically/ mean ± standard deviation (Apostolidis *et al.*, 2007)

Results

Antioxidant Activity

DPPH radical scavenging

The assay is grounded on the dimension of the scavenging capacity of antioxidants towards it. The odd electron of Nitrogen snippet in the DPPH is reduced by entering a hydrogen snippet from antioxidants to the corresponding hydrazine (Kedare and Singh, 2011), DPPH system has been extensively applied for estimating antioxidant exertion, still, its operations shough carried out bearing in mind the base of the system, and the need wherever possible to establish the stoichiometry for the quenching response, so that the antioxidant exertion may be related to the structure of the substrate patch. The system offers the advantages gas as being rapid-fire, simple, and affordable and provides first-hand information on the overall antioxidant Capacity of the test system. The grease paint antioxidant exertion attained by using the DPPH system is similar to trends set up using other styles. For a better understanding of the mechanisms involving the DPPH radical and im-

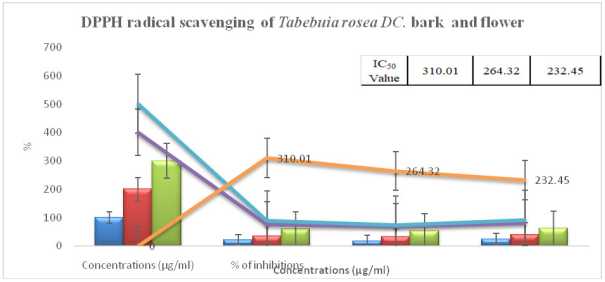

Fig. 1. Total antioxidant activity of *Tabebuia rosea* DC.

plicit antioxidants, it would be intriguing to characterize the response interceders and products. The half inhibition attention (IC 50) of factory Extract and ascorbic acid were bark 232.45 µg/ ml^{-1} FeNps Dinghy 310.01 µg/ ml and flower FeNps264.32 µg/ ml independently.

Total antioxidant activity of *Tabebuia rosea DC.*

The total antioxidant activity, which reflected the ability of the FeNps extracts to inhibit the bleaching of b-carotene, were measured and compared with that of the control which contained no antioxidant component. The b-carotene bleaching rates of the FeNps extracts are shown in Fig. 2. There were a decrease in absorbance values of B-carotene in the absence of vegetable extracts due to the oxidation of B-carotene and linoleic acid. The high absorbance values indicated that vegetable extracts possessed antioxidant activity (Arnao *et al*., 2001). The half inhibition concentration (IC 50) of plant Extract and ascorbic acid were 210.12 µg/ml^{-1} FeNpsbark 248.41 µg/ml and flower FeNps 235.47 µg/m; respectively.

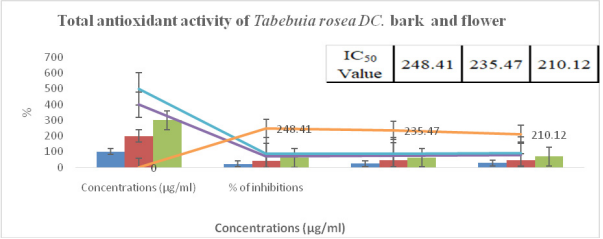

Fig. 2. DPPH radical scavenging of *Tabebuia rosea DC.*

Superoxide anion scavenging of *Tabebuia rosea* DC

The chloroform, ethanol, and aqueous extracts of *Tabebuia rosea DC* showed a concentration-dependent increase in the inhibition of superoxide generation, and the highest scavenging activity for O^{2-}, were observed at a concentration of 200 µg/ml for all three extracts (Lalhminghlui and Jagetia, 2018). The half inhibition concentration of Superoxide anion scavenging (IC-50) of Plant extract and ascorbic acid were 284.83 µg/ml^{-1} FeNps bark 344.47 µg/ml^{1} and flower NPS 303.54 µg/ml respectively.

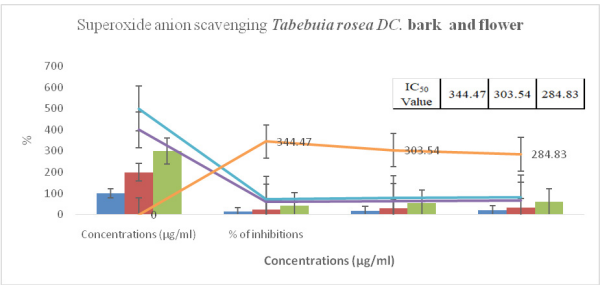

Fig. 3. Superoxide anion scavenging *Tabebuia rosea DC.*

Nitric oxide scavenging activity of *Tabebuia rosea DC.*

Nitricoxideisa free radical produced in mammal cells involved in the regulation of various physiological processes. However, excessive nitric oxide production is associated with several diseases such as carcinomas, youthful diabetes, multiple sclerosis, arthritis, and ulcer ativecolitis. The development of substance stop revent the overproduction of nitric oxide has become a new research target for the treatment of chronic inflammatory diseases. To neutralize this radical, no endogenous enzymatic scavenging pathway is present inside the body and most often it is neutralized by several endogenous molecules such as glutathione, melatonin, and antioxidants supplemented (Rani *et al*., 2022). The half inhibition concentration of Nitric oxide is a free radical produced (IC-50) of plant extract and ascorbic acid were 282.26 µg/ml^{-1}FeNpsbark 359.32 µg/ml and flower FeNPS 282.26 µg/ml respectively.

Reducing power assay of *Tabebuia rosea DC.*

For the measurement of the reductive ability, we investigated the Fe^{3+} Fe^{2+} transformations in the presence of *Tabebuia rosea* DC FeNPs extract following the standard method. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Like the antioxidant activity, the reducing power of *Tabebuia rosea **DC*** FeNPs extract increases with increasing concentration (Bhalodia *et al*., 2013). The half inhibition concentration of Reducing power assay (IC 50) of plant extract and ascorbic acid were 158.73 µg/ml^{-1}FeNpsbark 232.05 µg/ml and flower FeNPS 195.84 µg/ml^{-1}, respectively.

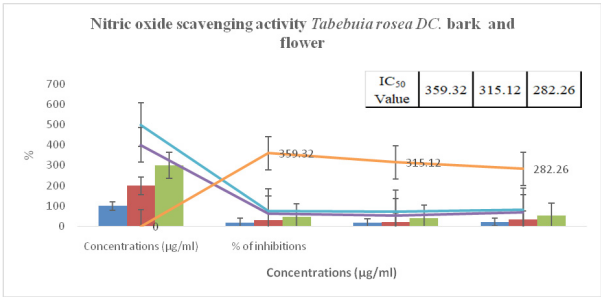

Fig. 4. Nitric oxide scavenging activity *Tabebuia rosea DC.*

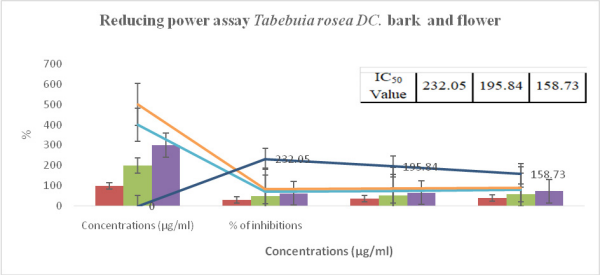

Fig. 5. Reducing power assay of *Tabebuia rosea* DC.

α-amylase inhibition assay of *Tabebuia rosea DC.*

The α-glucosidase and α-amylase inhibitory activity of FeNps extract, were confirmed in this study a maximum of 262.76µg/ml^{-1} and minimum of inhibition 272.42 µg/ml^{-1} and inhibition of α-amylase activity were observed at 309.23 µg/ml mg/ml concentration for the protein extracts from bark FeNps and flower FeNps and acarbose, respectively.

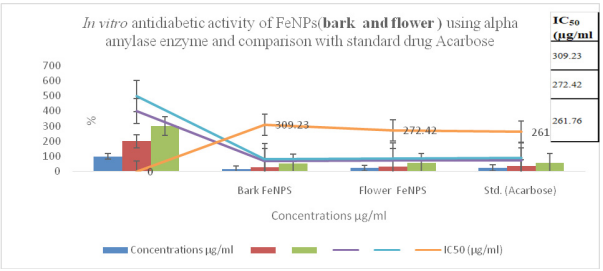

Fig. 6. *In vitro* antidiabetic activity of plant extract bark and flower

FeNPS using alpha amylase enzyme and comparison with standard drug Acarbose

A maximum of 309.23 µg/ml^{-1} and minimum of inhibition 261.76 µg/ml^{-1}. and inhibition of α-amylase activity were observed at 272.42 µg/ml^{-1}mg/ml concentration for the protein extracts from bark FeNps and flower FeNps and acarbose, respectively.

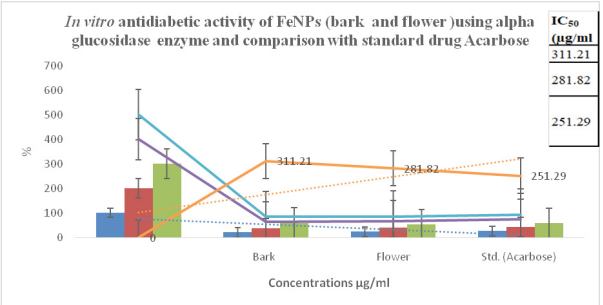

Fig. 7. Inhibition of alpha glucosidase activity by plant extract and standard Acarbose

The plant extract and standard Acarbose were found to be dose-dependent from 100 to 500 µg/ml^{-1} concentrations. A maximum of inhibition of α-amylase activity were observed at 500 µg/ml concentrate ion while a minimum of at 100 µg/ml for plant extract alpha glucosidase respectively and standard Acarbose respectively. The IC-50 were (bark and flower) 311.21, 281.82, vg/ml^{-1}and 251.29 µg/ml^{-1} for FeNPs extract standard Acarbose, respectively. The lowest IC-50 value has the highest antidiabetic activity.

Conclusion

The present study provides the first pharmacological insight into the antioxidant and antidiabetic potential of the selected medicinal *Tabebuia rosea DC*. FeNps powder showed high antioxidant capacity. These traditional medicinal plant extracts also reduced significantly α-amylase and α-glucosidase activities compared to the most common drug, acarbose, indicating that the polyphenols present in the extracts have the potential to reduce postprandial hyperglycemia by delaying carbohydrate digestion. The antidiabetic ability to inhibit α-amylase, α-glucosidase enzymes, and AGE are needed to be further explored using *in-vivo* experimental models to validate the findings in the present study.

Acknowledgement

The founders have nothing but praise for the professionals and specialized personnel at AVVM Sri Pushpam College, Bharathidasan University, for giving them a location to work. I'm writing to thank Dr. R. Manikandan, MSc, MCA., MPhil, Ph.D.,SET., my superior for his advice, helpful recommendations, and invigorating clarifications.

Conflict of Interest

None

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