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In vitro anti-oxidant and cytochrome P450 inhibitory activity of Pomegranate peel extract bio-assisted Strontium nanoparticles

Priyanka Sivasubramanian[1], Arjun Krishnan G[2], R.V.Geetha[1], Lakshmi Thangavelu, ParameswariRoyapuram Parthasarathy[3*]

[1]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai - 600 077, Tamilnadu, India.

[2]Department of Orthopaedics, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai, Tamil Nadu, India.

[3]Centre for Global Health, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai, Tamil Nadu, India.

***Corresponding Author**

Dr.Parameswari.R.P,

Associate Professor,

Centre for Global Health Research,

Saveetha Medical College and Hospital

Saveetha Institute of Medical and Technical Sciences,

Saveetha University, Chennai-602 105, Tamilnadu, India.

Email: parameswarirp.smc@saveetha.com

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ABSTRACT

Background

Metal and metal oxidenanoparticle synthesized using extracts of plants by green synthesis technology is biologically safe, cost-effective, and environment-friendly. Plants and their extracts have established the power to devour and accumulate inorganic metal ions from their neighbouring niche. As such, green synthesis is regarded as an important tool to reduce the destructive effects associated with the physical and chemical methods of synthesis for nanoparticles commonly utilised in laboratory and industry. In light of the given background, the present study aimed to analyse the in vitroanti-oxidant and cytochrome P450 inhibitory activity of strontium nanoparticles synthesised from pomegranate peel extract.

Materials and methods

Pomegranate peel extract was prepared and strontium oxide was mixed in the extract for the synthesis of the formulated nanoparticles. The antioxidant properties of the nanoparticles were evaluated using DPPH free radical scavenging assay and ABTS radical scavenging assay with ascorbic acid as standard. Further, the cytochrome P450 inhibitory activity was determined as well under in vitro conditions.

Result

Strontium nanoparticles synthesised from Pomegranate peel extract showed significant free radical scavenging and cytochrome P450 inhibitory activities. It was observed that the percentage inhibition of ABTS radical formation and percentage inhibition of DPPH free radical formation showed

dose-dependent effect along the increased concentration of nanoparticles. The CYP3A4 inhibitory activity of strontium nanoparticles showed significant increase with the increase in concentration of the nanoparticles.

Conclusion

Within the limits of the study, it can be concluded that Strontium nanoparticles synthesised from Pomegranate peel extract showed remarkable anti-oxidant and cytochrome P450 inhibitory activity. Further animal studies are needed to evaluate the efficacy of this nanocomposite.

KEYWORDS:*Strontium; Pomegranate peel extract; nanoparticle; plant extract; anti-oxidant; cytochrome P450*

INTRODUCTION

The development of functional foods and pharmaceuticals has greatly improved various aspects of health, including managing physical conditions, reducing dependence on synthetic antibiotics, and contributing to increased life expectancy. For centuries, plants have been a safe, effective, and sustainable source of natural antioxidants and free radical scavengers, largely due to their abundance of phenolic compounds such as phenolic acids, flavonoids, tannins, stilbenes, and anthocyanins [1]. These compounds are renowned not only for their strong antioxidant activity but also for their ability to inhibit cytochrome P450 enzymes, which are crucial in fighting a range of diseases and pathological conditions [2, 3]. Antioxidants, broadly defined, are substances that can delay or prevent oxidative damage to target molecules, with their primary feature being the capacity to neutralize free radicals [3] [12]. Cytochrome P450 (CYP) enzymes, membrane-bound haemoproteins, are essential for the detoxification of xenobiotics, as well as for maintaining cellular metabolism and homeostasis. The induction or inhibition of these enzymes is a key mechanism underlying many drug-drug interactions [4, 5] [13].

Punica granatum is one of the fruits that most attracted the interest of researchers for its high potential use in medicine and food industry. It is rich in several metabolites with anti-microbial, anti-cancer, anti-obesity, anti-diabetic, anti-ulcerogenic, and anti-hypertensive properties[6]. The above beneficial properties are not only limited to the edible part of fruit but also non-edible fractions viz., peel, seeds, flowers, bark, buds, and leaves.They come in huge quantities as by-products in pomegranate juice processing industries, constituting a great bioresource[7]. In particular, the peels represent about 50% of total fruit weight and are most often discarded as waste without any valorisation. Interestingly, pomegranate peel extract contains the highest concentration of phytochemicals, principally phenolic compounds family, such as tannins, ellagitannins, and anthocyanins, including ellagic acid and punicalagin[8]. These bioactive ingredients have proven antimicrobial propriety toward many pathogens and antiproliferative activity for different cell lines. Recently, phenols from hydro methanol pomegranate (*Punica granatum* L.) peel extract have shown interesting antihyperglycemic, antihyperlipidemic, and antioxidant properties[9, 10]. The smart recovery of pomegranate rind for production of extract could have multiple applications in different fields, such as biomedical, nutraceutical, and biocontrol [9-11].

A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometres in diameter. Among the nanoparticles, metallic nanoparticles have gained significant interest in the past few years due to their unique physical and chemical characters[12]. Strontium belongs to Group II metallic elements of the periodic table. Strontium (Sr) Nanoparticles, nanodots or nano powder are spherical or nanoflake high surface area metal particles. Green synthesis of strontium nanoparticles has gained popularity due to the economical and eco-friendly approach associated with it[13, 14].Among the other sources, plant mediated synthesis of nanoparticles is conferred due to the

presence of bioactive molecules that possess significant health benefits and one such plant includes pomegranate peel extract. In this present study, a simple and eco-friendly green synthesis method was utilised for the synthesis of strontium nanoparticles from pomegranate peel extract.Further, the study aimed on evaluating the anti-oxidant and cytochrome P450 inhibitory activity of strontium nanoparticles synthesised from Pomegranate peel extract.

MATERIALS AND METHODS

Sample preparation

Preparation of Pomegranate peel Extract

Fresh pomegranate peels were gathered, cleaned in running tap water, and then washed again with double-distilled water. The collected extract was boiled with 100 ml of distilled water at 60°C for about 20 minutes, to create the sample's aqueous extract. The extract was then brought to room temperature, filtered through paper using a filter, and utilised in the study.

Preparation of strontium nanoparticles (PP-Sr NPs)

Strontium oxide was mixed in the extract for the creation of the nanoparticles. This solution was constantly agitated with a magnetic stirrer. After the combination had completely dissolved, the solution was vigorously stirred for 5 to 6 hours at roughly 150°C. After cooling the solution to room temperature, the supernatant was removed and discarded. After thorough washing, the resultant was centrifuged twice at 4500 rpm for 15 minutes before being dried at 80°C for 7–8 hours.

Antioxidant Assays

Free radical scavenging activity of Pomegranate peel mediated strontium nanoparticles (PP-Sr NPs) was determined by DPPH and ABTS radical scavenging assays described earlier[15][16].

DPPH Free radical scavenging assay

In DPPH radical scavenging assay, 10µL different concentrations of the synthesised nanoparticles (5, 10, 20, 40, 80 & 160µL) was added to 190µL of DPPH (150µM prepared in ethanol). The reaction mixture was shaken thoroughly and incubated in dark for 30 min at 37°C. After incubation, the absorbance was measured at 517 nm using Biotek synergy H4 hybrid microplate reader, USA. The reaction mixture without the nanoparticle was used as control and ascorbic acid was used as standard. The % inhibition of DPPH free radical formation was calculated as follows: [(Control - Test)/Control] *100

ABTS radical scavenging assay

The ABTS (2,2'-azino-di [3-ethylbenzthiazoline sulfonate]) assay was performed as follows: 10µL different concentrations of the synthesised nanoparticles (5, 10, 20, 40, 80 & 160µL) was added to 10µL of metmyoglobin and 150µL of 2mM ABTS. The reaction was initiated by adding 40µL of H202 (441µM). The reaction mixture without the test drug was kept as control and ascorbic acid was used as standard. The absorbance was read at 690 nm using Biotek synergy H4 hybrid microplate reader, USA. The % inhibition of ABTS radical formation was calculated as follows: [(Control - Test)/Control] *100

CYP34A inhibitory activity

Briefly, various concentrations of the synthesised nanoparticle, potassium phosphate buffer, CYP450 reagent and substrate 7-Benzyloxy-4-trifluoromethylcoumarin (BFC) were added to a 96-well plate. The mixtures were pre - incubated for 20 min at room temperature. The reaction was started by a mixture of reconstituted substrate and NADP+ and incubated at room temperature for 30-60 min. The reaction was stopped by the Tris-HCl buffer, pH 10.5. The fluorescent intensities of the products were measured by Biotek synergy H4 hybrid microplate reader using an excitation and emission wavelength of 405 nm and 460 nm, respectively.

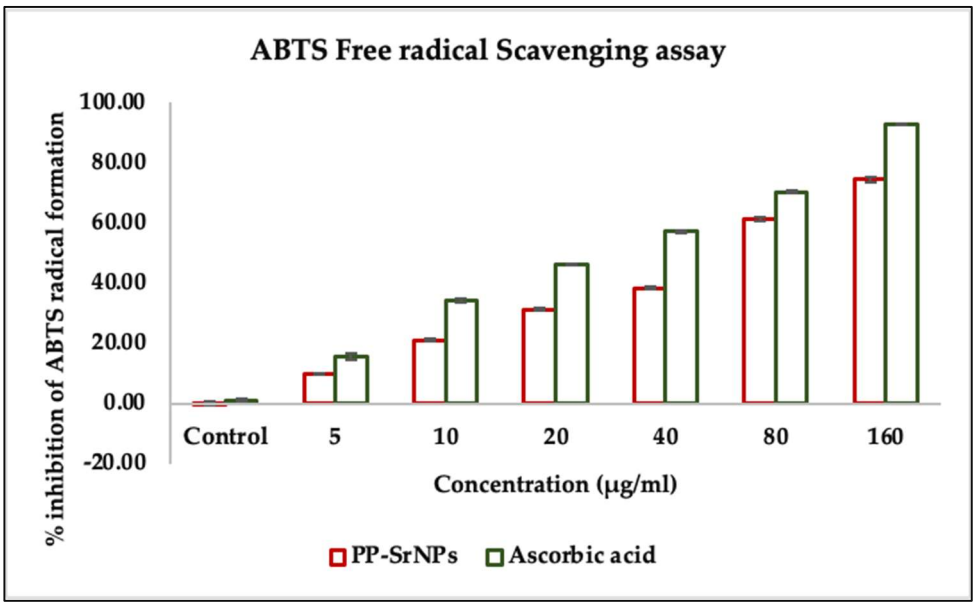
RESULTS

Effect of PP-Sr NPs on ABTS free radicals' formation

The ability of PP-Sr nanoparticles to inhibit ABTS radical formation was evaluated across a concentration range of 5 to 160µg/mL. The results demonstrated a concentration-dependent increase in % inhibition, with values ranging from -0.03% at the lowest concentration to 74.49% at the highest concentration (160 µg/mL). This suggests that as the concentration of PP-Sr NPs increases, their antioxidant activity and ability to neutralize free radicals also improves significantly.When compared to the standard antioxidant, ascorbic acid, which showed a % inhibition range from 1.05% to 92.76% over the same concentration range, it is evident that PP-Sr NPs exhibit strong antioxidant potential, particularly at higher concentrations. Although ascorbic acid demonstrated superior inhibition across all concentrations, PP-Sr NPs still exhibited a substantial free radical scavenging capacity, particularly noticeable at concentrations of 80 µg/mL and above, where the % inhibition exceeded 60%.

These results suggest that while PP-Sr NPs may not be as potent as ascorbic acid, they still offer considerable antioxidant activity, especially at higher concentrations, and could serve as effective free radical scavengers in various biomedical applications. Further investigation into the mechanism of action and comparative studies with other antioxidants could help elucidate their full potential.

Figure 1: Effect of PP-Sr NPs on ABTS free radical formation - ABTS Free radical scavenging assay. Data expressed as Mean±SEM (n = 3).



Effect of PP-Sr NPs on DPPH free radicals' formation

The antioxidant activity of PP-Sr NPs was assessed using the DPPH free radical scavenging assay, with inhibition of DPPH radical formation measured across a concentration range of 5-160µg/mL. The % inhibition of DPPH radical formation increased in a dose-dependent manner, starting from 0% at the lowest concentration and reaching 81.12% at 160µg/mL. In comparison, the standard antioxidant, ascorbic acid, exhibited a stronger inhibition, with % inhibition values ranging from 0.56% at the lowest concentration to 94.78% at 160µg/mL.While PP-Sr NPs demonstrated a significant capacity to scavenge DPPH free radicals, their efficacy was slightly lower than that of ascorbic acid across all concentrations.

At lower concentrations, the difference between the two was relatively small, but at higher concentrations (160µg/mL), ascorbic acid showed a more pronounced inhibitory effect. Nonetheless, the progressive increase in % inhibition for PP-Sr NPs indicates their potential as effective antioxidants, especially at higher concentrations, though further optimization may enhance their radical scavenging activity to match or exceed that of standard antioxidants like ascorbic acid.

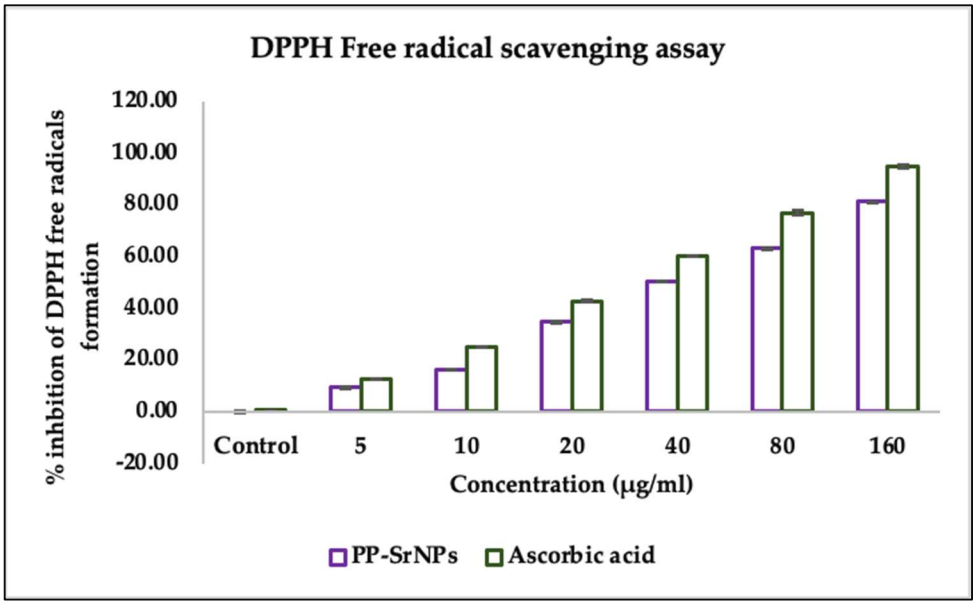


Figure 2: Effect of PP-Sr NPs on DPPH free radical formation - DPPH Free radical scavenging assay. Data expressed as Mean±SEM (n = 3).

Effect of PP-Sr NPs on Cytochrome P450 isoform, CYP3A4 activity

The inhibition of CYP3A4 activity by PP-Sr nanoparticles (NPs) was evaluated across a concentration range of 10–160µg/mL. The results showed a concentration-dependent inhibition of the CYP3A4 enzyme, with the percentage inhibition ranging from -0.07% to 70.54%. At lower concentrations, such as 10 µg/mL, inhibition was minimal (-0.07%), indicating negligible effects on enzyme activity. However, as the concentration increased, there was a marked rise in inhibition, with 9.24% at 20 µg/mL and progressively higher inhibition at 30.90% for 80µg/mL. The highest level of inhibition, 70.54%, was observed at the maximum concentration of 160µg/mL.These results suggest that PP-Sr NPs significantly inhibit CYP3A4 activity in a dose-dependent manner, with higher concentrations exhibiting stronger inhibitory effects. This indicates that PP-Sr NPs have the potential to modulate cytochrome P450 enzyme activity, particularly CYP3A4, which could have implications for drug metabolism and potential drug-drug interactions. Further investigation into the mechanistic pathways of this inhibition and its pharmacological relevance is warranted.

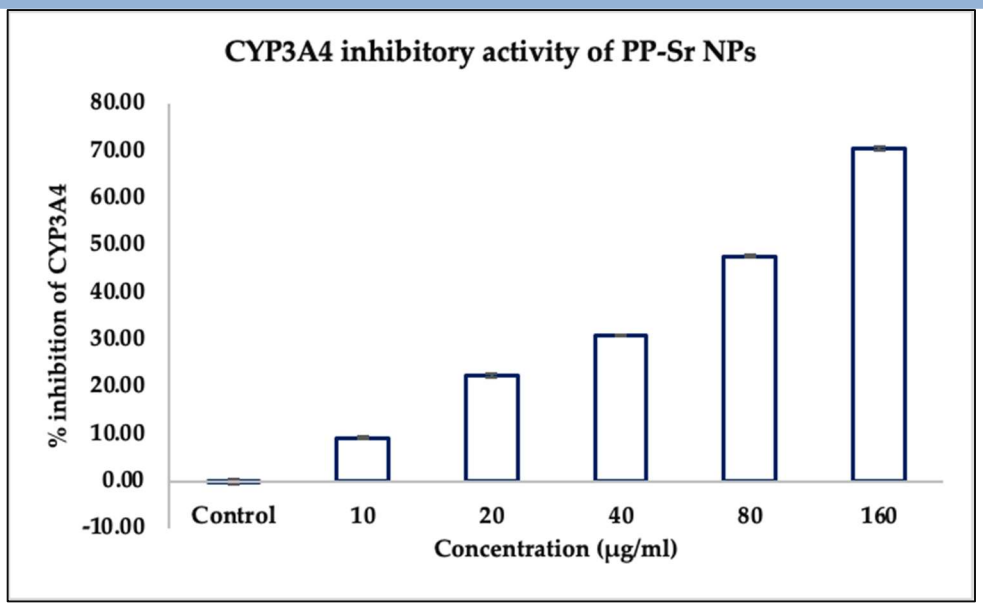


Figure 3: Effect of PP-Sr NPs on Cytochrome P450 inhibitory activity - CYP3A4inhibitory assay. Data expressed as Mean±SEM (n = 3)

DISCUSSION

The present study demonstrated the inhibitory effects of PP-Sr nanoparticles (NPs) on cytochrome P450 isoform CYP3A4 activity. A clear dose-dependent inhibition was observed, with CYP3A4 activity decreasing progressively as the concentration of PP-Sr NPs increased from 10 µg/mL to 160 µg/mL. At 160 µg/mL, the highest inhibition level reached 70.54%, indicating a significant impact on the enzyme's function. This strong inhibition of CYP3A4 suggests that PP-Sr NPs have the potential to alter drug metabolism, as CYP3A4 is a major enzyme involved in the oxidation of xenobiotics and endogenous compounds.

These findings are in line with previous research on natural products and nanoparticles that affect cytochrome P450 enzymes. For instance, it has been well-established that dietary polyphenols exhibit selective inhibition of cytochrome P450 enzymes, particularly in the arachidonic acid cascade, highlighting structure-dependent selectivity and potency [17, 18]. This suggests that the structure of PP-Sr NPs might similarly play a crucial role in their interaction with the CYP3A4 isoform. Additionally, studies have shown that silver nanoparticles also exhibit significant inhibitory effects on hepatic cytochrome P450 enzymes in rats, indicating that metallic nanoparticles, in general, can alter the enzyme's activity [19]. Similarly, the inhibition of human cytochrome P450 enzymes by metallic nanoparticles, including silver and gold, has been well-documented, supporting the potential of nanoparticles to modulate enzyme activity [20]. The inhibition of multiple human P450 enzymes by the constituents of *Ginkgo biloba* extract further corroborates the role of natural products and nanoparticles in the suppression of P450 enzyme activity [21,22].

The observed inhibition of CYP3A4 by PP-Sr NPs in this study may have significant implications for drug-drug interactions. Since CYP3A4 is responsible for the metabolism of a wide range of pharmaceuticals, its inhibition by PP-Sr NPs could potentially lead to reduced drug clearance, enhanced drug bioavailability, and, consequently, increased risk of toxicity. This warrants careful consideration when exploring the therapeutic applications of PP-Sr NPs in clinical settings.In addition to their effect on CYP3A4 activity, the free radical scavenging activity of PP-Sr NPs was also noteworthy in this study.

The nanoparticles demonstrated potent antioxidant activity, as evidenced by their ability to scavenge free radicals in vitro. This dual role of PP-Sr NPs—acting as both enzyme inhibitors and antioxidants—parallels findings from other studies on natural compounds. The potent antioxidant activity of polyphenols and other plant-derived compounds has been linked to their ability to inhibit cytochrome P450 enzymes, suggesting a shared mechanism involving redox modulation [23]. The antioxidant properties of PP-Sr NPs may contribute to their ability to regulate oxidative stress and modulate enzymatic activity, providing a potential therapeutic advantage in conditions associated with oxidative damage and altered drug metabolism.

CONCLUSION

In summary, the present study highlights the significant inhibitory effects of PP-Sr NPs on CYP3A4 activity and their strong free radical scavenging activity. These findings align with previous reports on the enzyme-modulating potential of natural products and nanoparticles. Further studies are needed to explore the structural features of PP-Sr NPs that govern their selectivity and potency in inhibiting CYP3A4, as well as to assess their broader pharmacological implications in vivo.

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CONFLICT OF INTEREST

The authors declared no conflict of interest pertaining to the study.

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