ORIGINAL ARTICLE

In vitro evaluation of anti-inflammatory, anti-oxidant activity of pomegranate peel extract mediated calcium sulfate nano particles

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ABSTRACT

Introduction: Pomegranate peel is considered a reservoir of biologically active compounds, the presence of which provides anti-inflammatory and antioxidant properties to peel extracts. Calcium sulfate is considered an ideal bone-augmenting material, and in the present study, pomegranate peel extract-mediated calcium sulfate nano particles (PPE CaSo4 NPs) were synthesized and their anti-inflammatory and antioxidant properties were evaluated. This study aimed to evaluate the biological effects of PPE CaSo4 NPs, with a focus on their anti-inflammatory and antioxidant properties. The objectives were to green - synthesize PPE CaSo4 NPs, analyze their optical characteristics using UV-visible spectra analysis, and subsequently evaluate their antioxidant and anti-inflammatory activities.

Materials and Methods: UV-visible spectra analysis was employed to investigate the optical characteristics and surface morphology, such as the size and shape of PPE CaSo4 NPs synthesized at different time intervals, which were characterized using a Scanning Electron Microscope. Anti-inflammatory activity was evaluated using bovine serum albumin denaturation assay (BSA), and egg albumin denaturation assay (EA) was compared with diclofenac sodium as a standard. Antioxidant activity was measured using 2,2 Diphenyl -1- Picryl hydraxylhydrate assay (DPPH), hydrogen peroxide radical scavenging assay (H2O2), and Ferric Reducing Antioxidant power assay (FRAP). Comparison made with ascorbic acid as the standard.

Results: Anti-inflammatory activity was observed at all concentrations of PPE CaSo4 NPs, and there was no significant difference between the test material and the standard p>0.05. A significant difference was found for the antioxidant activity between PPE CaSo4 NPs and the standard in concentrations of 10 µl, for DPPH, 10 µl and 20 µl for H_2O_2 ($p<0.05$) between the concentrations of 30, 40, and 50, and there was no significant difference between the test material and the standard in all three tests conducted.

Conclusion: The study concluded that the PPE CaSo4 NPs have Anti-inflammatory and Antioxidant activities and are concentration-dependent.

KEYWORDS:
Anti-inflammatory, Antioxidant, Calcium sulfate, Pomegranate peel extract, Flavonoids

INTRODUCTION

For more than a century calcium sulfate (CS) has been employed as an alloplastic material for bone regeneration, establishing itself as a prominent element in the field of regenerative materials. Unlike numerous commercially available biomaterials used clinically, calcium sulfate possesses unique properties.[1] It is a biocompatible and bioresorbable substitute and a cost-effective naturally occurring inorganic substance. Calcium sulfate has osteoconductive properties, which increases its use for regenerative purposes.[2] Osteoconductive materials act as a matrix that provides support to the ingrowth of bone in the presence of bone-forming cells in the host. Additionally, it can restore the original anatomical features and structural properties of bone. Studies have shown that when CS is implanted, calcium ions bind to phosphate ions found in body fluids, resulting in the formation of calcium phosphate, which exhibits osteoconductive properties. Calcium phosphate also reduces the pH locally, causing bone demineralization. This process exposes growth factors such as Bone morphogenic protein (BMP) transforming growth factor-β (TGF-β) and others stored within the bone matrix, thereby promoting bone growth.[3] When CS was used to fill large bony defects, it was found that the material resorbed completely from the grafted site without leaving any trace elements. The resorption rate of calcium sulfate was comparable to the rate at which new bone formed and facilitated the migration of osteoprogenitor cells into the area.[4] The formation of new blood vessels plays a critical role in all regenerative processes, including bone regeneration. It is triggered by high metabolic activity and reduced oxygen levels at the injury site. The occurrence of angiogenesis can be confirmed by assessing microvascular density at the regenerative site.[5,6] Ruhaimi et al. examined the osteogenic potential after combining CS with various other graft materials. They concluded that CS can cause angiogenesis, which was confirmed by the presence of multiple microvessels without any signs of inflammation. They inferred that angiogenesis can be considered as an added property of CS

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that aids in osteogenesis.[7] Investigations also reported that CS acts as a direct source of calcium, and resorption leaves the calcium phosphate lattice, which promotes osteogenic activity.[8,9]

Extracts of pomegranate peel are reported to have an abundance of phytochemicals and vitamins that are responsible for anti-inflammatory and antioxidant properties, punicalagin A & B, ellagic acid, and gallic acid present in the pomegranate have anti-inflammatory effects by inhibiting lipopolysaccharides -stimulated macrophages that down-regulate COX-2 protein release. As a result, it decreases the levels of pro-inflammatory mediators, such as inducible nitrous oxide (iNO), PGE-2, interleukins, and Reactive oxygen species (ROS).[10] Inflammation can cause bone destruction by pannus formation. Ellagic acid present in pomegranate inhibits inflammation by decreasing pro-inflammatory cytokines IL - 1β, Tumor Necrosis Factor (TNF-α,) and IL17 and increasing serum levels of anti-inflammatory cytokines IL-10 and interferon γ (IFN-γ).[11,12] The anti-inflammatory properties of a specific type of PPE were evaluated and a significant anti-inflammatory effect of PPE on the expression of cyclooxygenase-2 was confirmed.13 In obese patients, extracts derived from pomegranate peels showed anti-inflammatory effects by significantly reducing inflammatory markers [malondialdehyde (MDA), IL-6, and hypersensitive-C reactive protein (hs-CRP)].[14] A randomized controlled trial conducted in patients with rheumatoid arthritis found that there was a significant decrease in disease activity following 8 weeks of supplementation with PPE.[15]

Research has shown that extracts derived from pomegranate fruit can protect against osteoarthritis. This protection is achieved through mechanisms such as increased cartilage stiffness, physical fitness, decreased levels of enzymes that break down cartilage, and enhancing the body's antioxidant defense system.[16] According to Spilmont, a diet supplemented with pomegranate peel extract enhanced bone mineralization in osteoporotic rat models by upregulating osteogenic transcription factors and stimulating alkaline phosphatase activity (ALP).[17] Additionally, he also revealed that the punicic acid (conjugated linolenic acid) rich pomegranate seed oil causes osteoblastic stimulation and inhibits osteoclastic suppression and thus prevents bone loss in an osteoporotic mice model.[18] The antioxidant potential of aqueous extracts derived from by-products of pomegranate and grapes was evaluated. The total phenolic content of the pomegranate by-product was five times higher than that of red grapes.[19]

Owing to its anti-inflammatory and antioxidant properties, pomegranate fruit and its biomolecules can be recommended as a functional food option to mitigate bone loss and related disorders. This suggests that incorporating pomegranate into the diet may help prevent bone-related issues. Since CS is an osteoconductive material widely used in bone augmentation and pomegranate extracts promote osteogenesis due to the presence of biologically active compounds and its anti-inflammatory and antioxidative properties, this study aimed to evaluate the anti-inflammatory and antioxidant properties of pomegranate peel extract mediated calcium sulfate nanoparticles.

The objectives were to green – synthesize PPE CaSo4 NPs, to analyze their optical characteristics of pomegranate peel-mediated calcium sulfate nano particles using UV-visible spectra analysis, and subsequently evaluate its antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS

Preparation of Pomegranate peel extract

The pericarp of fresh Ganesh variety pomegranate fruits was separated and immersed in diluted Koparo Clean vegetable and fruit wash for 15 min and then air-dried. The dried peel was ground into a coarse powder using a multi-milli-machine made of SS 304.

Next, 2 g of peel powder was mixed with 100 mL of distilled water using a magnetic stirrer (Remi 5MLH). The mixture was heated in a heating mantle set at 60 to 80ºC for 15 - 20 min. After heating, the mixture was filtered through Whatman No. 1 filter paper. After filtration, the extract was further condensed to a volume of 5mL

Green synthesis Pomegranate peel extract mediated calcium sulphate nano particles (PPE CaSo4 NPs)

Green synthesis of PPE- mediated Caso4 was performed by dissolving 0.5gm of Caso4 (99% pure calcium sulfate (VITSZEE ®) in 25 ml of distilled water, 25 ml of filtered PPE was added, and the mixture was stirred magnetically at 600 RPM for 24h. After 24h, the green synthesized PPE-mediated CaSo4 nano particles were collected and the supernatant was discarded.

Characterization

The green-synthesized calcium sulfate nano particles were characterized using a UV-visible spectrophotometer to study the optical characteristics, while the surface morphology, such as size and shape, was characterized using a Scanning Electron Microscope.

Anti-inflammatory activity

Egg Albumin Denaturation Assay (EA)

A 5 ml solution was prepared by mixing 2.8 ml of freshly made pH-6.3 phosphate-buffered saline with 0.2 ml of Hen's egg albumin extraction. PPE CaSo4 NPs were synthesized at various concentrations ranging from 10µL to 50µL. Diclofenac sodium was used as the positive control. Subsequently, the mixtures was heated in a water bath at 37°C for 15 min. After cooling the samples to room temperature, absorbance was measured at 660 nm.

Bovine Serum Albumin Denaturation Assay (BSA)

In this study, the anti-inflammatory effects of pomegranate peel extract-mediated calcium sulfate Nano particles were evaluated following a modified protocol based on Muzushima and Kabayashi's method. To initiate the assay, 0.45 mL of 1% aqueous bovine serum albumin was mixed with 0.05 mL of PPE CaSo4 NPs of varied fixation (10µL, 20µL, 30µL, 40µL, and 50µL). The pH of the mixture was adjusted to 6.3 using a small amount of 1N hydrochloric acid. Subsequently, the samples were incubated at room temperature for 20 min, followed by heating at 55°C in a

water bath for an additional 30 min. After cooling the samples, the absorbance was measured at 660 nm using a spectrophotometer. Diclofenac sodium was used as the benchmark and dimethyl sulfoxide (DMSO) served as the control.

The percentage of protein denaturation was determined utilizing the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of sample}\times}{\text{Absorbance of control}}$$

Antioxidant activity

Three techniques were used to evaluate the antioxidant activity of PPE CaSo4 NPs, two of which, DPPH and H_2O_2, were dependent on PPE CaSo4 NPs's ability to scavenge free radicals. The iron-reducing capacity provided the basis for the third method, called FRAP.

The assay used a commercially available free radical 2,2 diphenyl- 1-picryl hydrazyl hydrate (DPPH) which is soluble in methanol, and the antioxidant activity was measured by the decrease in absorbance at 515 nm. Different concentrations of PPE CaSo4 NPs (10µL, 20µL, 30µL, 40µL, 50µL) were mixed with 450µl of 50 mM Tris-HCl buffer (pH 7.4) and 1 ml of 0.1 mM DPPH in methanol. The mixture was then incubated for 30 min. afterwards, the reduction in DPPH free radical levels was determined by measuring the absorbance at 517 nm. Butylated hydroxytoluene (BHT) was used as control. Percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of test sample} \times 100}{\text{Absorbance of control}}$$

H_2O_2 assay

The H_2O_2 scavenging power of green-synthesized PPE CaSo4 NPs was assessed. 40 mM H_2O_2 solution was prepared in phosphate buffer (pH 7.4). Solution of the test sample PPE CaSo4 NPs and a standard sample of ascorbic acid at varying concentrations (10, 20, 30, 40, and 50µg/mL) were individually added to 0.6 mL of H_2O_2 solution. After 10 min of incubation in the dark place, the absorbance of the reaction solution was spectrophotometrically measured at 230 nm. Vitamin C was used as the standard. The percentage of H_2O_2 scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

FRAP ASSAY

Reagents for FRAP assay:

a)Acetate buffer 300 mM pH 3.6: Weigh 3.1 g sodium acetate trihydrate add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6- tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) FeCl3. 6 H2O: (M.W. 270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b, and c in a ratio of 10:1:1 just before testing. The standard was FeSO4. Seven H2O: 0.1 - 1.5 mM in methanol. All the reagents were purchased from Merck (Germany) company.

Procedure

To perform the FRAP (Ferric Reducing Antioxidant Power) assay, an FRAP solution (3.6 mL) was combined with 0.4 mL of distilled water and incubated at 37°C for 5 min. Subsequently, the solution was mixed with various concentrations of PPE$CaSo_4$NPs (10µL, 20µL, 30µL, 40µL, 50µL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. To construct the calibration curve, five concentrations of FeSO4 7 H2O (0.1, 0.4, 0.8, 1, 1.12, and 1.5 mM) were used, and their corresponding absorbance values were measured, mirroring the procedure for the sample solutions.

RESULTS

Optical characteristics

UV-visible spectral analysis was employed to investigate the optical characteristics of pomegranate peel mediated calcium sulfate nanoparticles synthesized at various time intervals. Notably, absorption peaks were discerned at 350 nm after 48h of synthesis and at 340 nm for both 1 h and 24hs of synthesis. The 350 nm peak strongly indicates the presence of calcium sulfate nanoparticles, with UV absorption commonly linked to electronic transitions within the nanoparticles. This specific peak signifies nanoparticles of distinct sizes and shapes, corresponding to a unique bandgap energy (Fig 1).

SEM

The SEM image of the calcium sulfate nanoparticles synthesized using pomegranate peel extract revealed crucial insights into their morphology and size distribution. The nanoparticles exhibited a predominantly spherical morphology with well-dispersed particles uniformly distributed across the field of view, indicating effective stabilization by the pomegranate peel extract. The sizes of the nano particles ranged from approximately 50 to 150 nm, demonstrating a relatively narrow size distribution. This consistency suggests the effectiveness in controlling the nucleation and growth of the nanoparticles during synthesis. The clean, well-defined surfaces of the nanoparticles indicate the purity and high quality of the synthesis process with no visible impurities or irregularities. The pomegranate peel extract functioned effectively as both a reducing and capping agent, resulting in stable and uniform nanoparticles. The uniform size distribution and spherical morphology of these calcium sulfate nanoparticles suggests their potential applicability in biomedical applications, drug delivery systems, and as fillers in composite materials. The eco-friendly synthesis method using pomegranate peel extract not only effectively produces these nanoparticles, but also aligns with sustainable and green chemistry principles (Fig 2).

Anti-inflammatory activity

In EA, the inhibition caused by PPE CaSo4 NPs at 10µL concentration was (52.00% ±1.05), and 55% ± 1.56% for the standard. For the 50 µl concentration, it was 77.00% ±1.15 and 81% ±1.69%, respectively. For BSA, the percentage of inhibition with10µl concentration was (41.40% ±11.08), and

Table I: Comparison between Anti-inflammatory activity obtained in different concentrations of PPE CaSo4 NPs using EA & BSA assay

Concentration	% of inhibition	N	Mean	Std. Deviation	Std. Error Mean	p-value
10 µL	PPECaSo4NPs	10	52.0000	1.05409	0.33333	0.388
EA	Standard	10	55.0000	1.56347	0.49441	
20 µLl	PPE CaSo4 NPs	10	61.0000	1.05409	0.33333	1.000
EA	Standard	10	64.0000	1.05409	0.33333	
30 µL	PPE CaSo4 NPs	10	65.0000	1.05409	0.33333	0.591
EA	Standard	10	69.0000	1.15470	0.36515	
40 µL	PPE CaSo4 NPs	10	69.0000	2.10819	0.66667	0.160
EA	Standard	10	72.0000	1.05409	0.33333	
50 µL	PPE CaSo4 NPs	10	77.0000	1.15470	0.36515	0.118
EA	Standard	10	81.0000	1.69967	0.53748	
10 µL	PPE CaSo4 NPs	10	41.4000	11.08753	3.50619	0.087
BSA	Standard	10	46.7000	1.56702	0.49554	
20 µL	PPE CaSo4 NPs	10	52.4000	11.52003	3.64295	0.185
BSA	Standard	10	59.6000	2.79682	0.88443	
30 µL	PPE CaSo4 NPs	10	63.4000	11.76813	3.72141	0.139
BSA	Standard	10	71.4000	2.59058	0.81921	
40 µL	PPE CaSo4 NPs	10	68.9000	10.19204	3.22301	0.124
BSA	Standard	10	75.7000	2.11082	0.66750	
50 µL	PPE CaSo4 NPs	10	76.3000	9.34582	2.95541	0.354
BSA	Standard	10	83.3000	3.77271	1.19304	
	'*' p-value <0.05 shows statistical significance					

Table II: Comparison between Antioxidant activity of PPE CaSo4 NPs obtained in DPPH, H2O2 and FRAP

Concentration	% of inhibition	N	Mean	Std. Deviation	Std. Error Mean	p-value
10 µL	PPE CaSo4 NPs	10	64.3040	0.01174	0.00371	0.019
DPPH	Standard	10	66.2500	0.00471	0.00149	
20 µL	PPE CaSo4 NPs	10	73.6410	0.00876	0.00277	0.549
DPPH	Standard	10	78.5200	0.00667	0.00211	
30 µL	PPE CaSo4 NPs	10	83.7820	0.00919	0.00291	0.651
DPPH	Standard	10	85.6330	0.01160	0.00367	
40 µL	PPE CaSo4 NPs	10	86.1210	0.00738	0.00233	0.558
DPPH	Standard	10	88.6820	0.00919	0.00291	
50 µL	PPE CaSo4 NPs	10	91.1220	0.00919	0.00291	0.558
DPPH	Standard	10	93.1510	0.00738	0.00233	
10 µL	PPE CaSo4 NPs	10	49.4500	0.00667	0.00211	0.007*
H2O2	Standard	10	51.1000	0.11547	0.03651	
20 µL	PPE CaSo4 NPs	10	53.9800	0.00667	0.00211	0.036*
H2O2	Standard	10	56.9000	0.20548	0.06498	
30 µL	PPE CaSo4 NPs	10	64.7600	0.00667	0.00211	1.000
H2O2	Standard	10	64.5600	0.00667	0.00211	
40 µL	PPE CaSo4 NPs	10	75.7100	0.00667	0.00211	0.064
H2O2	Standard	10	75.5300	0.06360	0.02011	
50 µL	PPE CaSo4 NPs	10	87.4200	0.00667	0.00211	1.000
H2O2	Standard	10	87.2200	0.00667	0.00211	
10 µL	PPE CaSo4 NPs	10	67.3200	0.01054	0.00333	0.591
FRAP	Standard	10	72.9800	0.01155	0.00365	
20 µL	PPE CaSo4 NPs	10	74.1800	0.01491	0.00471	0.361
FRAP	Standard	10	76.8400	0.01054	0.00333	
30 µL	PPE CaSo4 NPs	10	77.9000	0.17638	0.05578	0.050
FRAP	Standard	10	81.3100	0.01054	0.00333	
40 µL	PPE CaSo4 NPs	10	81.0900	0.02108	0.00667	1.000
FRAP	Standard	10	85.8400	0.02108	0.00667	
50 µL	PPE CaSo4 NPs	10	86.3800	0.01054	0.00333	0.391
FRAP	Standard	10	90.8900	0.02357	0.00745	
	'*' p-value <0.05 shows statistical significance					

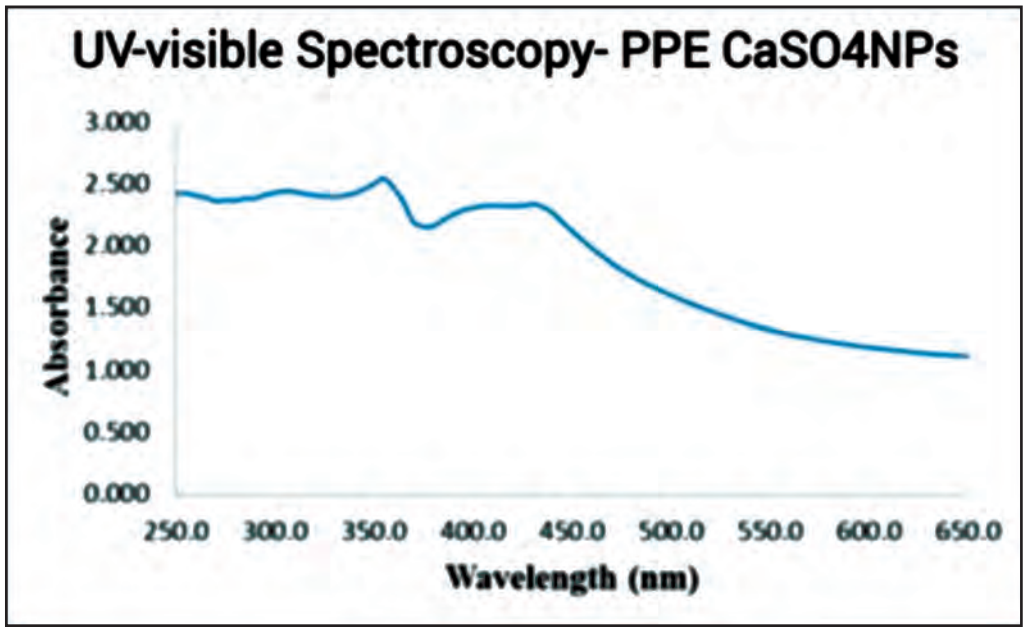

Fig. 1: UV-visible spectroscopy –PPE CaSo4NPs

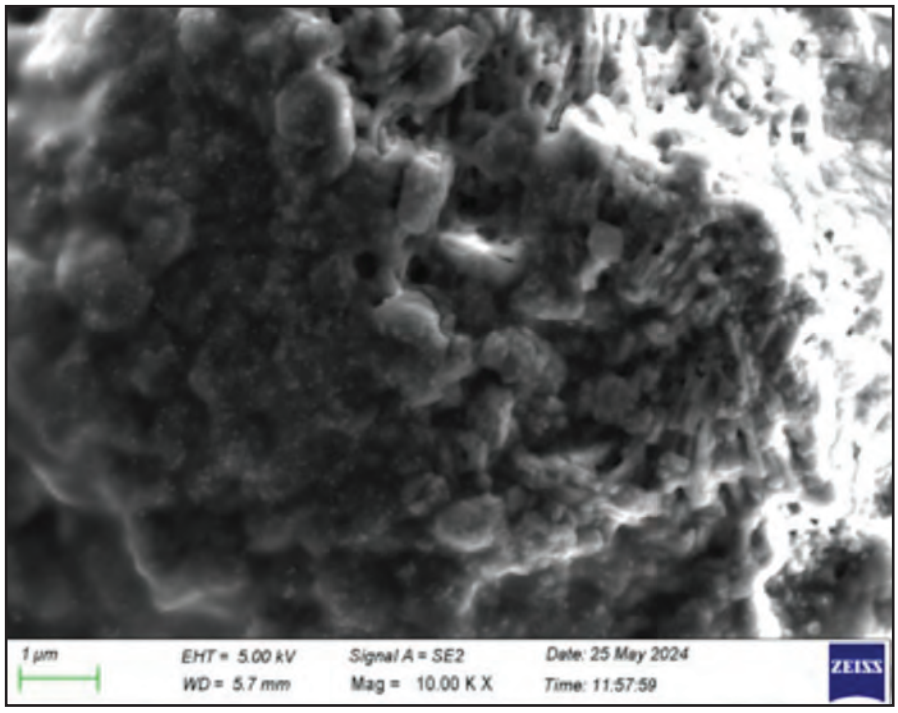

Fig. 2: SEM PPE CaSo4 NPs

for the standard it was 46.70% ± 1.56. For the 50 µl concentration, it was 76.30% ± 9.34 and 83.30% ±3.72%, respectively. It was observed that in tests of EA and BSA all concentrations, there was no significant difference between the test material and the standard ($p>0.05$). Comparisons between the mean values of different concentrations observed in EA and BSA were performed using ANOVA. Each test (EA, BSA) showed characteristic independence, and it can be inferred that in all the tests, if the same pattern is observed, it can be considered reliable ($p< 0.05$). Multiple comparisons were made using Tukey's HSD on values obtained in EA and BSA. Different concentrations were mutually compared and found that on comparing, all concentrations showed significant differences $p<0.05$. (Table I)

Antioxidant activity

The antioxidant activity of PPE CaSo4 NPs observed in DPPH ranged from 64.30% ±.011 to 91.12% ±.009 in the

experimental group and 66.25% ±.004 to 93.15% ±.007 with the standard. For H_2O_2, the values were 49.45% ±.006 to 87.42% ±.006 for the experimental group and 56.90% ±.20 to 87.22% ±.006 for the standard group. For FRAP the values were 67.32% ±.01 to 86.38% ±01 and 72.98% ±.01 to 90.89% ±.02 respectively. A significant difference was found between PPE CaSo4 NPs and the standard in concentrations of 10 µl, for DPPH, 10 µl and 20 µl for H_2O_2 ($p<0.05$) between the concentrations of 30, 40, and 50, and there was no significant difference between the test material and the standard in all three tests conducted. In other words, at these concentrations, in all three tests, there was equivalence between the standard and test groups ($p>0.05$).

Comparisons between different concentrations of PPE CaSo4 NPs in DPPH, H_2O_2, and FRAP were performed using ANOVA. A significant difference was observed at all concentrations in all three tests ($p<0.05$). Multiple comparisons were made using Tukey's HSD for values obtained for DPPH, H_2O_2, and FRAP. Different concentrations were mutually compared, and all concentrations were found to significantly different ($p<0.05$). (Table II)

DISCUSSION

Calcium sulfate has been used as an effective bone augmentation material for the past 100 years. It is considered an ideal material for bone regeneration as it provides calcium ions to the augmented site, acts as a resorbable scaffold for bone growth, stimulates osteoblastic activity, acts as a vehicle for growth factors and drugs, and is biocompatible. Researchers found that compared to other implanted regenerative materials, CS has rapid and complete resorption, and the rate of resorption is almost equivalent to the rate at which new bone is created.[20,21] The particle size of materials can influence bone healing, and nano-sized calcium sulfate dihydrate is reported to have good tissue compatibility, increased biodegradable properties, increased angiogenesis, and increased bone induction. Therefore, calcium sulfate nano particles were used.[22]

Biologically active compounds that contribute to the anti-inflammatory and antioxidant properties are concentrated in the peel of pomegranate, making it a significant reservoir.[23] In the present study, pomegranate peel of the Ganesh variety was used to prepare the aqueous extract, and PPE-mediated calcium sulfate nano particles were green synthesized. The anti-inflammatory and antioxidant properties of green-synthesized pomegranate peel extract-mediated calcium sulfate nanoparticles (PPE CaSo4 NPs) were evaluated.

In this study, egg albumin denaturation assay and bovine serum albumin denaturation assay were used to measure the anti-inflammatory properties of PPE CaSo4 NPs. Protein denaturation causes inflammation. Evaluation of PPECaSo4 on the anti-inflammatory activity showed that the extract inhibited protein denaturation. In both assays of EA and BSA at all concentrations, there was no significant difference between the test material and the standard, that is, diclofenac sodium $p>0.05$. Thus, the anti-inflammatory activity of the test material was the same as that of the control. Different concentrations were mutually compared and found that on comparing, all concentrations showed significant differences $p<0.05$. Anti-inflammatory activity increased as the concentration increased. Therefore, it can be concluded that the anti-inflammatory activity was concentration-dependent. The results obtained for PPE CaSo4 NPs were comparable to those of commercial drugs used, indicating that PPE CaSo4 NPs possesses anti-inflammatory properties similar to those of the control, that is diclofenac sodium.

The extracts of pomegranate peel, juice, flowers, and seeds were found to have high polyphenol concentrations. The gut bacteria convert these polyphenols into urolithins, which have anti-inflammatory properties.[24] Pohl et al reported that Pomegranate's biologically active components, which are present in the fruit's peel, juice, and extracts, affect the expression of a protein that signals inflammation in cancer cells.[25] Tannin, and punicalagin present in the pomegranate extracts decreased the expression of cyclooxygenase–2 (COX-2) responsible for the production of prostanoids that induce inflammation.[26] The action of ellagic acid found in pomegranate peel extract was reported by Marín, Ellagic acid inhibited the inflammation by decreasing the inflammation mediating compounds COX-2 and iNOS and blocking the cell signalling pathway in the intestine.[27] The use of polyphenol-rich pomegranate fruit extract or compounds derived from it for the treatment of inflammatory diseases by suppressing basophils and mast cell activation was reported by Rasheed Z et al.[28] Free radicals that generate oxidative stress are what causes inflammation, and the antioxidant properties of pomegranates can get rid of the free radicals and lessen the inflammation. Pomegranate also has antibacterial Properties, and its phytochemicals can reduce inflammation caused by microorganisms. As a result, it is useful for the treatment inflammations caused by microbes. Pomegranate phytochemicals have multiple bioactivities; they can influence a variety of inflammatory variables and promote healing.[29,30] The observations of the above-mentioned studies substantiate the results obtained in this study.

The antioxidant activity was measured using DPPH, H_2O_2, and FRAP assays ,and ascorbic acid was used as the staanderd. At concentrations of 10 µl for DPPH, 10 µl and 20 µl for H_2O_2 significant difference was found between PPE CaSo4 NPs and the standard ($p<0.05$) at higher concentrations of 30, 40, and 50 µl; there was no significant difference between the test material and the standard in all the three tests conducted, that is DPPH, H_2O_2 and FRAP. This indicated equivalence between the standard and test groups at these concentrations ($p>0.05$).

It was also found that antioxidant activity increased as the concentrations increased from 10µL to 50 µL. A significant difference was found in all concentrations in all three tests ($p<0.05$) Indicating that the antioxidant properties increased with an increase in concentration, that is the antioxidant activity was dose-dependent and the PPE CaSo4 NPs can provide the same antioxidant as the standard. This finding is similar to the findings of previous studies that concluded that pomegranate peel extracts have high concentrations of biologically active compounds responsible for the antioxidant activity, and the concentration of the

compounds depends on the method of extraction and the cultivar, and the antioxidant activity increases as the concentration of the peel extract increased.[31-35] It can also be stated that the addition of calcium sulfate did not decrease the antioxidative properties as the values obtained were comparable with the standard used.

CONCLUSION

The distinctive biochemical profile of pomegranate and the presence of more than 124 bioactive components confirm its anti-inflammatory and antioxidant properties. The antioxidant activity of pomegranate peel extract-based calcium sulfate was comparable to that of the control, and was directly related to the concentration. The capacity of pomegranate peel extract-based calcium sulfate to suppress protein denaturation proved its concentration- dependent anti-inflammatory potential. According to this study, pomegranate peel extract-mediated calcium sulfate Nano particles possess anti-inflammatory and antioxidant characteristics that vary in concentration. The findings of this investigation will advance our knowledge of these features. Hence, thorough testing is required before pomegranate and its bioactive chemicals can be employed as therapeutic agents, either alone or in combination, to treat a variety of degenerative disorders such as osteoporosis, rheumatoid arthritis, and bone regeneration.

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