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CHITOSAN NANOPARTICLES FROM *PERSEA AMERICANA* SEEDS: ANTIDIABETIC FORMULATIONS & BIOACTIVITY ASSESSMENT

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Keywords:

Chitosan, *Persea americana*, Nanoparticle, Herbal formulation, Bioassay

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ABSTRACT: Avocado *(Persea americana)* seeds are an excellent source of antioxidants, and phenolic compounds were used to create green synthesis nanoparticles that were then incorporated into formulations for solid herbal tablets. The process of ionic gelation is used to synthesize biopolymer chitosan nanoparticles. The characterization was done by UV and FTIR spectroscopy. FTIR spectroscopy showed that chitosan nanoparticles included many functional groups. Pre- and post-compression experiments were conducted on a herbal tablet formulation made using the direct compression method. Biological activities like antidiabetics, antioxidant, anti-inflammatory, anti-bacterial and anti-fungal studies were carried out using *in-vitro* method. Anti-diabetic activity resulted in an appreciable percentage inhibition of α-Amylase (99.82%), which is comparable with the standard metformin (86.05%). Antioxidant activity measured using DPPH method demonstrated the scavenging activity of herbal formulations (67.98%) and nanoparticles (58%), surpassing standard ascorbic acid (18%). The herbal formulation exhibited the largest percentage inhibition of anti-inflammatory action (77.79%) as determined by the human red blood cell membrane stabilization technique, and nanoparticles (70.38%) exceeding standard drug diclofenac (43.7%). The tablets that were developed demonstrated significant anti-bacterial and anti-fungal characteristics. This led to the development of a zone of inhibition that measured 20-25 mm for *S. aureus*, 23-27 mm for gram-negative bacteria such *E. coli* and *S. typhi*, and 20-25 mm for *C. balantis*. MTT assay performed to detect the cytotoxicity of fibroblast cells indicated cell viability higher than 100%. Avocado seed extract-based formulations have diverse bioactivities, making them potential candidates for diabetes management, antioxidant therapy, anti-inflammatory treatments, and combating infections.

INTRODUCTION: *Persea americana*, also known as avocadowhich belongs to the family Lauraceae of evergreens, is a native of the Americas. It is medium in size. It produces large, pear-shaped berries with green or yellow flesh and a single, large seed. The seed is rich in fatty acids, antioxidants, and phytochemicals, and has been used for various medicinal purpose [1, 2].

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However, the seed also contains some toxic compounds, such as persin and tannins that may cause adverse effects in humans and animals if consumed in large amounts. Therefore, the safety and efficacy of avocado seed consumption need further research and evaluation [3, 4].

Some of the phytochemicals found in avocado seeds are abscisic acid, phenolic compounds, flavonoids, saponins, and alkaloids. These substances may possess a range of biological properties, including anti-inflammatory, antidiabetic, antioxidant, antibacterial, and antifungal properties. However, the exact mechanisms, active components, pharmacokinetics,

and clinical efficacy of these phytochemicals are still unclear and require more investigation [4]. An abundant substance found in crab shells, chitin, is deacetylated to produce chitosan, a naturally occurring polymer. In the realm of nanomedicine, chitosan has garnered a lot of interest because of its mucoadhesivity, biocompatibility, biodegradability, and capacity to create nanoparticles with a variety of medications and biomolecules [5].

Chitosan nanoparticles can enhance the stability, solubility, and bioavailability of drugs, as well as protect them from degradation and premature release. Moreover, chitosan nanoparticles can modulate the drug release profile according to the pH and enzymatic conditions of the target site [6].

The objective of this research is to create chitosan nanoparticles as an innovative medication delivery method for seed extract of *Persea americana*. Hypothesize that chitosan nanoparticles can encapsulate the seed extract and deliver it to the desired site of action, while minimizing the toxicity and enhancing the bioactivity of the extract. The ionic gelation process was utilized to manufacture chitosan nanoparticles. Ionic gelation is a simple, fast, and cost-effective method that does not require organic solvents or high temperatures. It can encapsulate the *Persea americana* seed extract and protect it from degradation and premature release [7]. This study assesses the cytotoxicity of the chitosan nanoparticles and seed extract in addition to their antidiabetic, antioxidant, anti-inflammatory, antibacterial, and antifungal properties. Chitosan nanoparticles demonstrate their potential as a promising tool for drug delivery and nanomedicine.

MATERIALS & METHOD:

Preparation of *Persea americana* (Avocado) Seed Powder: The pulp from freshly obtained avocados from a local market was removed, and the seeds were collected. After rinsing the seeds under water for eliminating any remaining fruit residues and patting them dry, they were left exposed to air until completely dried. The seeds were cut into smaller pieces using knife for easy grinding, ground to the desired texture using a blender or food processor, and dried in an oven at 60°C to reduce moisture. The seeds that had been powdered were kept for later usage at room temperature in an airtight container.

Synthesis and Characterization of Chitosan Nanoparticles from Avocado Seed Extract: The ionic gelation method was employed to produce chitosan nanoparticles. To 80 ml of 1% acetic acid, 0.1 g of chitosan was added. A magnetic stirrer was used to agitate this mixture. Subsequently, the linking agent, sodium tripolyphosphate (2%), was added and thoroughly mixed on the magnetic stirrer at room temperature for 60 minutes until an opalescent colour was developed [8].

Ionic Gelation Technique for Loading Plant Extract onto Chitosan Nanoparticles: The seed extract underwent decoction for concentration. The resulting extract was added dropwise to a chitosan solution on a magnetic stirrer for 2 hours. After centrifugation at 1000rpm for 10 minutes, the obtained pellet was suspended in phosphate buffer saline and centrifuged again at 10,000rpm for 20 minutes. After washing thrice with distilled water, the nanoparticle obtained was transformed into powder using a ROTA evaporator. The functional property and morphology of the nanoparticle were then analyzed using FTIR and SEM, respectively [8].

Formulation of Herbal Tablet Containing *Persea Americana* Seed Extract: Tablets made from *Persea americana* seed extract nanoparticles were formulated and evaluated [9]. The process involved mixing the seed extract nanoparticle with other excipients and compressing them into tablets using the direct compression method carried out at the pharmacy laboratory of Sterling Institute of Pharmacy, Navi Mumbai. Three batches (F1, F2, and F3) each weighing 500 mg, were prepared with slight variations in the excipient composition, as outlined in the table.

TABLE 1: THE COMPOSITION OF FORMULATIONS OF TABLETS

Ingredients	Quantity per tablet (Mg)		
	F1	F2	F3
Terminalis Chebula	200	200	200
Persea americana seed extract nanoparticle	100	50	75
Ginger powder	10	20	15

Papain enzyme	50	100	75
Cumin seed powder	20	20	20
Salt	10	10	10
Microcrystalline cellulose	30	30	30
Ethyl cellulose	20	-	30
Carbopol	-	20	-
Dibasic calcium phosphate	30	20	15
Stearic acid	20	20	20
PEG-400	10	10	10
Weight per tablet	500	500	500

Blend Preparation: The quantity of excipient was calculated for a batch of 25 tablets each of diameter 10.0 mm, round, standard concave punch size, and corresponding tablet weight, all excipients were weighed and passed through a sieve of mesh number 44 and mixed for 2 minutes. Lubricated granules were compressed using the suitable punch to produce tablets with sufficient hardness [10].

Preformulation Studies:

Optical Microscopy for Determination of Physical form: Particles, placed on a clean slide with mineral oil and observed under a 100x magnification microscope.

Loss on Drying: The substance to be tested was sieved using mesh number 44 British Standard in Surveying (BSS). The sample-filled petri dish was baked for one hour at 105 degrees Celsius. The weight of the Petri plate containing the cooled sample was measured once it had cooled to room temperature.

Angle of Repose: A short stem glass funnel, secured by a ring clamp on an iron stand, was positioned over graph paper to collect powder. 25 grams of the sieved sample was poured into the funnel forming a cone-shaped pile. The diameter 'd' of the resulting circle was measured, and the radius calculated. Using two rulers, the pile's height 'h' was measured and recorded for calculation.

Bulk Density: 25 grams of sample was weighed and passed through the sieve of mesh number 44, after that, it is transferred into a 100-graduated cylinder. The powder level was levelled before compacting and the unsettled apparent volume was read followed by the calculation of bulk density g/ml.

Tapped Density: The same cylinder used for measuring bulk density underwent 10, 500, and 1250 taps, and the corresponding volumes were recorded. A check ensured that the difference between these volumes did not exceed 1 ml.

Evaluation of Prepared Tablets: Tablet dimension (thickness and diameter): Using a Vernier calliper, the average diameter and thickness of each tablet were determined.

Weight Variation: Each tablet was weighed separately, and the average weight was determined. The deviation and percentage of weight variation were then calculated by comparing the weight of each tablet to the weight of the average tablet.

Hardness: The tablets were positioned in the jaws of the Monsanto hardness tester and subjected to diametrical force until they broke. Initial and final readings were recorded, and the difference between the two readings was taken as the tablet hardness.

Friability: Tablets were weighed, then placed inside the friability drum and rotated 100 times. After removal, the tablets were weighed again to calculate the weight loss.

Disintegration: Tablets were inserted into the six tubes of the basket, each with a disc. Using water at room temperature, the disintegrator tester apparatus was operated. The time was recorded both before the start and at the completion of full tablet disintegration.

Prepared herbal tablet formulation studies were compared with a marketed herbal tablet, Maharishi Ayurveda Triphala tablet.

***In-vitro* Antidiabetic Studies by DMSO Method:** To 0.5M tris HCL buffer (0.2 ml of pH 6.9) containing 0.01 M $CaCl_2$, 2mg of starch was suspended (substrate solution) and boiled for 5 minutes, followed by preincubation in an incubator for 5 minute. Nanoparticle, three batches of tablet, and metformin tablet were dissolved in DMSO to

get varying concentrations (10, 20, 40, 60, 80, and 100 mg/ml). Each concentration (0.2 ml) was added to the substrate solution tube. To the tube containing the tablets and nanoparticles, 0.1 ml of alpha-amylase in tris HCL buffer (2 units/ml) was also added. The reaction was conducted at room temperature in the incubator for 10 minutes. To stop the reaction, 0.5 ml of 50% acetic acid was added to each tube, followed by centrifugation at 3000 rpm for 5 minutes at 4ºC. Resulting supernatant's absorbance was measured at 540 nm on a spectrophotometer [11]. The standard medication was alpha-amylase inhibitor metformin, and the alpha-amylase inhibitory activity was determined.

Antioxidant Activity by DPPH Assay: Ascorbic acid, nanoparticles, and herbal tablets were prepared at ascending concentrations (5 concentrations from 10-50 mg/ml). As a diluent, one milliliter of distilled water was introduced to tubes containing the test and standard.

Additionally, 1 ml of DPPH reagent was added to each tube containing the test and standard. The reaction mixtures were then incubated in a dark environment for 30 minutes due to DPPH's light sensitivity. Absorbance was measured at 517nm, and optical density (OD) was recorded, with ethanol serving as a control [12]. Subsequently, the inhibition ratio was calculated based on these measurements.

Human Red Blood Cell Membrane Stabilization Technique:

***In-vitro* Anti-Inflammatory Activity:** Whole blood from a healthy volunteer was used to make a 10% v/v RBC suspension in saline solution. Regarding the reaction mixture, phosphate buffer, RBC suspension, a hypotonic saline solution and different concentrations of nanoparticles (31.25, 62.5, 125, 250µg/ml) herbal tablets, and (diclofenac) was used as the standard were combined. The reaction mixture was incubated in the incubator for 30 minutes, followed by absorbance measurement at 570nm. Two milliliters of distilled water mixed with phosphate buffer and red blood cell suspension was used as a control. The percentage protection was calculated [13].

Anti-bacterial Activity by agar well Diffusion Method: The agar well diffusion method was used to evaluate the herbal tablet's antibacterial properties. For the assay, nutrient broth (1.3 grams) and agar powder were dissolved in 100 ml of distilled water. In an aseptic environment, a culture suspension was created with optical density 0.1. Nanoparticles and tablets, intended for studying antibacterial properties, were dissolved in 1 ml distilled water. If the dissolution was incomplete after slight boiling and centrifugation, resulting in partial activity, the supernatant was collected, and the pellet discarded. In a sterile suspension, 3 ml sterile saline was mixed with bacterial strains. The mixture was then placed into sterile Petri dishes and allowed to solidify, divided into sections for tablets 1, 2, 3, and a hole for the positive control. Four wells were symmetrically made using a borer, and the extract was poured into them. Plates were incubated, and after 24 hours, the formation of the zone of inhibition was observed [14].

Antifungal Activity: The agar well diffusion method was used to assess the herbal tablet's antifungal efficacy. A fungal suspension (20 µl), diluted was mixed with prepared sterile media and evenly spread onto Petri plates. The plates were left to dry thoroughly before wells were created using a stainless-steel borer. Then, 200 microliters of herbal tablet solution (500 mg in 1 ml distilled water) were poured into the wells, including a well for a positive control comparison. Incubation of the plates at room temperature occurred for 24-72 hours, and the antifungal activity was assessed by measuring the zone of inhibition, using amphotericin b as the standard antibiotic [15].

MTT Assay for *In-vitro* Toxicity Testing: MTT (3-(4,5- dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide) assay on cell culture of chick embryo fibroblast. The method is based on tetrazolium salt reacting with mitochondrial dehydrogenase that generates insoluble blue-violet crystals. Chick embryo extract preparation and cell line induction involved dissection of 14-day-old fertilized eggs on Day 1. After isolating, mincing, treating with trypsin, and centrifuging for 10 minutes at 3000 RPM, the thigh region was examined. After discarding the supernatant, the cell pellet was subjected to two washes using 1X PBS (pH 6.4), and a Neubauer hemocytometer was used to count the cells.

On Day 2, the media was replaced in nanoparticle and herbal tablet plates. Day 3 involved adding 40µl of MTT reagent to the media, incubating for 3 hours, then adding 50µl of DMSO, ensuring proper mixing without vigorous shaking.

Absorbance was read at 570nm using an ELISA microplate reader, after which the plate was rocked for 30 minutes before a final absorbance reading [16].

RESULTS & DISSCUSSION:

Chitosan Nanoparticle Biosynthesis: Chitosan nanoparticles, created via ionic gelation, had their supernatant removed and processed into a powdered.

Ultraviolet-visible Spectroscopy (UV-Vis):

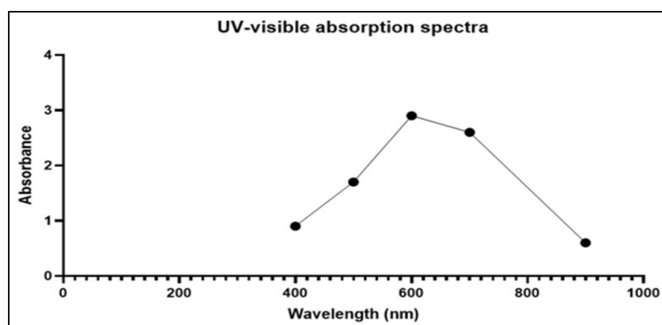

FIG. 1: UV-VIS ABSORPTION SPECTRA OF CHITOSAN NANOPARTICLE

Fourier Transform Infrared Spectroscopy: The study involved determining changes in the functional group of nanoparticles using Fourier transform infrared spectroscopy to analyze alterations in the overall composition.

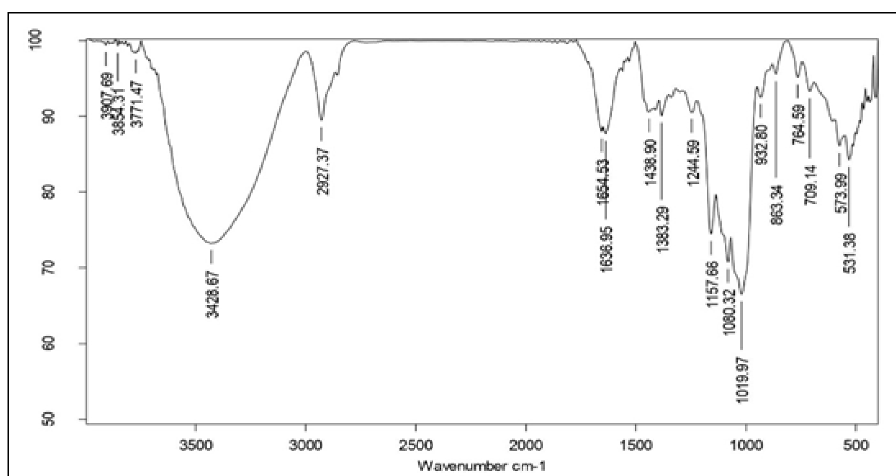

FIG. 2: FTIR ANALYSIS OF CHITOSAN NANOPARTICLE

TABLE 2: TABLE CONTAINING THE ANALYSIS RESULT OF GRAPH OBTAINED FROM FTIR

Sr. no.	Absorption peak	Appearance	Group	Compound Class
1	3428.67	Strong, Broad	O-H Stretching	Alcohol
2	2927.37	Medium	O-H Stretching	Carboxylic acid
3	1654.53	Medium	C-H Bending	Aromatic compound
4	1636.95	Medium	C=C Stretching	Alkene
5	1438.90	Medium	C-H Bending	Alkane
6	1383.29	Weak	C-H Bending	Aldehyde
7	1244.59	Medium	C-N Stretching	Amine
8	1157.66	Strong	C-N Stretching	Amine
9	1080.32	Strong	C-N Stretching	Amine
10	1019.97	Strong	C-O Stretching	Ether
11	932.80	Weak	C-H Bending	-
12	863.34	Weak	C-H Bending	1,2,3 trisubstituted
13	764.59	Weak	C-H Bending	Monosubstituted
14	709.14	Weak	C=C Bending	Alkane
15	573.99	Medium	C-Cl Stretching	Halo-compound
16	531.38	Medium	C-Br Stretching	Halo-compound

The overlapping peaks in the FTIR spectrum indicate the presence of similar functional groups, while the sharpness of peak correlates with their quantity. A very strong peak at 3428.7cm^{-1} indicates the presence of alcohol, phenol, or primary/secondary amine. The presence of a very

weak peak is assigned to Alkyne stretching, and the medium peak is primarily due to stretching of the C-C bond, primary amine, or N-H band.

Herbal Tablet Pre Formulation Study: The chemical and physical properties of the ingredients prior to formulation were revealed by the formulation study that was carried out. The results of the formulation study indicated varying observations. The loss on drying (LOD) value for the granules of the API sample recorded 32%, surpassing the acceptable range. The Hausner ratio of the granules from the API sample was 1.24 and compressibility index stood at 19.36%, representing an acceptable value. However, the angle of repose (tan θ) displayed favourable results across all batches (F1= 23.74º, F2= 27.02º, F3= 23.74º), indicating excellent flow properties and non-stickiness.

Herbal Tablets Post Formulation Studies: The evaluation of uncoated herbal tablets (500mg) from three separate batches (F1, F2, and F3) prepared by direct compression method was conducted to ascertain tablet dimensions. F1 demonstrated a thickness of 5.47 mm and a diameter of 10.53 mm, F2 showed a thickness of 5.66 mm and a diameter of 10.72 mm, while F3 exhibited a thickness of 5.50 mm and a diameter of 10.73 mm. In comparison, the standard Maharishi Ayurveda Triphala tablet had a thickness of 5.86 mm and a diameter of 8.1 mm. The tablet diameter should not surpass 22 mm and ideally should be 8 mm or less, while its thickness must not compromise stability, hardness, or friability.

Assessment of weight variation revealed that F1, F2, and F3 displayed average weights of 0.52g (% deviation: ± 8%), 0.505g (% deviation: ± 3%), and 0.49g (% deviation: ± 15%) respectively, with an acceptable deviation limit of ± 5% for uncoated tablets weighing over 500mg. Hardness measurements indicated values of 3 kgf for F1, 7 kgf for F2, and 3 kgf for F3, whereas the acceptable hardness range for tablets typically ranges between 5-10 kgf. Friability tests showed F1 with 0.64%, F2 with 0.99%, and F3 with 1.36%, while the acceptable friability range is yet to be specified. Disintegration time assessments revealed that F1 disintegrated in 15 minutes, F2 in 14 minutes, and F3 in 8 minutes, meeting the Indian Pharmacopoeia (IP) standard of less than 15 minutes for tablet disintegration.

Alpha Amylase Inhibition Activity: In evaluation of alpha-amylase inhibition by Metformin, nanoparticles, and herbal tablets (F1, F2, F3) at a concentration of 100 µg/ml, F1 & F2 shows highest inhibitory activity 99.82% and, metformin shows second highest inhibitory activity of 86.05%, F3 shows 79.19% inhibitory activity and nanoparticles shows inhibitory activity of 42.78%. F1 and F2, the herbal tablets demonstrated potential against alpha-amylase activity and F3 can be a good comparison for metformin for alpha amylase inhibitory activity. This underscores their potential as inhibitors. A regular medication used to treat type 2 diabetes is metformin, which increases insulin sensitivity and reduces the liver's synthesis of glucose [17]. However, metformin has some side effects such as gastrointestinal disturbances, lactic acidosis, and vitamin B12 deficiency [18]. F1 and F2 may have fewer or milder side effects than metformin, as they are natural products [19]. Hypoglycemiahas been reported after the extract has been administered. Furthermore, administration of the *Persea Americana* extract to rats led to an elevation of phospho-PKB expression in the soleus muscle. In the process of absorbing glucose, this enzyme's activation causes the GLUT-2 molecule to move from the cytoplasm to the cell membrane [20].

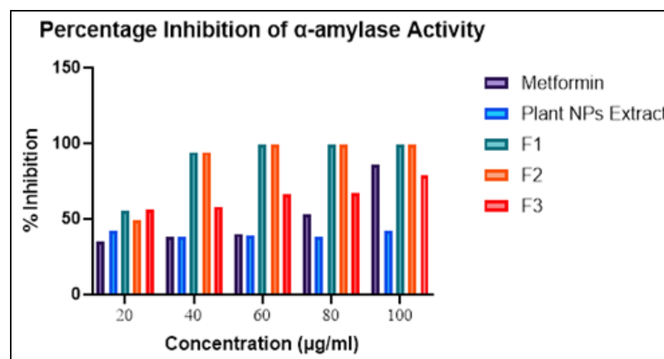

FIG. 3: GRAPH SHOWING THE PERCENTAGE ALPHA-AMYLASE INHIBITORY EFFECT OF NANOPARTICLE, METFORMIN (STANDARD DRUG), F1, F2 & F3

Antioxidant Activity: The antioxidant activity of herbal tablets (500 mg) of *Persea americana* seed (F1, F2, F3), nanoparticles of *Persea americana* seed extract and standard ascorbic acid was evaluated by DPPH assay. The percentage of DPPH radical scavenging is used to express a sample's antioxidant activity. Among the samples

tested, F3 showed the highest antioxidant activity (67.98%), followed by nanoparticles (58%), F1 (42.5%), ascorbic acid (18%) and F2 (18%).

The results indicate that the *Persea americana* seed extract and its nanoparticles have higher antioxidant potential than the standard ascorbic acid, and that the formulation of the herbal tablets affects their antioxidant activity.

F3, which contains the highest amount of *Persea americana* seed extract, exhibits the best antioxidant performance among the herbal tablets. The nanoparticles, which have a smaller size and a larger surface area, also show a higher antioxidant activity than the bulk extract [21].

The *Persea americana* seed extract's phenolic components are capable of offering DPPH radicals electrons or hydrogen atoms to neutralize them thus, providing antioxidant qualities.

Avocados are rich source of flavonoids, Because of their ability to react with free radicals and reactive substances, which render damaged cell tissue inactive, the flavonoids function as reactive oxygen species [20].

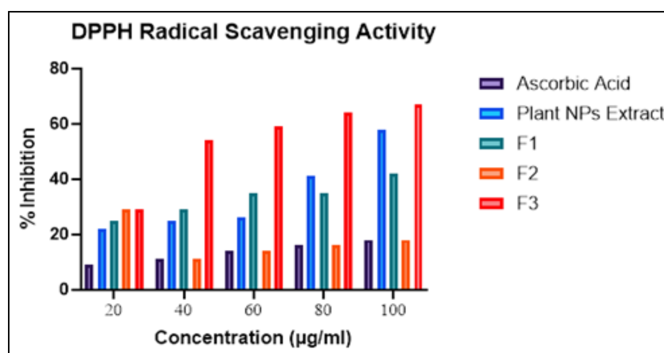

FIG. 4: THE PLOT OF DPPH RADICAL SCAVENGING ACTIVITY AS A FUNCTION OF CONCENTRATIONS OF NANOPARTICLE, ASCORBIC ACID (STANDARD), F1, F2 & F3

Anti-inflammatory Activity: The anti-inflammatory activity of herbal tablets (500 mg) of *Persea americana* seed (F1, F2, F3), plant extract nanoparticles and standard diclofenac was evaluated by the HRBC membrane stabilization method. This method measures the ability of the test substances to prevent the lysis of human red blood cells (HRBC) induced by heat or hypotonicity [22]. Using various concentrations (31.25, 62.5, 125 µg/ml) of the test substances, the percentage of hemolysis inhibition was computed.

TABLE 3: HUMAN RED BLOOD CELL MEMBRANE STABILIZATION TECHNIQUE: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITYAT VARIOUS CONCENTRATIONS (31.25, 62.5, 125 µG/ML)

Concentration (µg/ml)	% of inhibition by				
	Diclofenac	Plant extract nanoparticles	F1	F2	F3
31.25	30.52	63.5	71.42	49.26	30.52
62.5	30.86	69.32	75.5	59.44	30.86
125	43.7	70.38	77.79	59.79	43.7

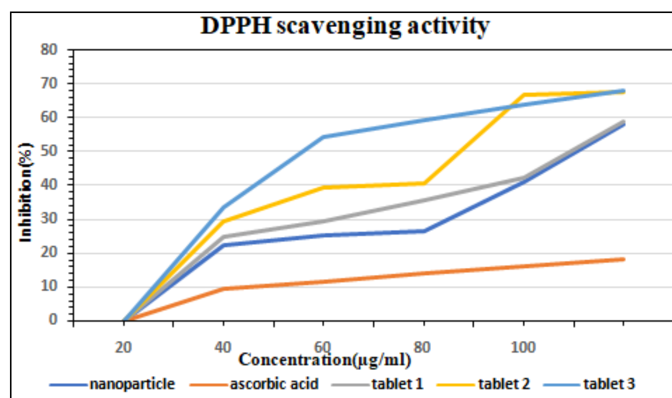

FIG. 5: THE PLOT OF DPPH RADICAL SCAVENGING ACTIVITY AS A FUNCTION OF CONCENTRATIONS OF NANOPARTICLE, ASCORBIC ACID (STANDARD), TABLET 1 AS F1, TABLET 2 AS F2 &TABLET 3 AS F3

The results showed that all the test substances exhibited anti-inflammatory activity in a concentration-dependent manner. Among them, F1 had the highest activity, followed by plant extract nanoparticles, F2, diclofenac and F3. F1 showed significantly higher activity than diclofenac, the standard anti-inflammatory drug, at all concentrations. Plant extract nanoparticles also showed higher activity than diclofenac, except at the lowest concentration. F2 showed moderate activity, while F3 showed similar activity to diclofenac. Based on these results, *Persea americana* seed and seed extract nanoparticles may be helpful in treating inflammatory diseases due to their strong anti-inflammatory properties.

More research is required to determine the active ingredients and potential mechanisms of action underlying the anti-inflammatory properties. Avocado oil has been shown to have beneficial

effects on hepatic markers in rats fed sugar, which had changed hepatic markers. Avocado oil's antioxidant composition makes it a promising candidate for use as a metabolic syndrome preventative factor because it considerably lowers LDL, TG, and VLDL levels without changing HDL levels. Also, the hs-CRP levels were lowered with avocado oil, suggesting a partial reversal of inflammatory processes [23].

Antibacterial Activity: The antibacterial activity of herbal tablets made of *Persea americana* seed extract (F1, F2, F3) was evaluated by the agar well diffusion method. With this technique, zones of inhibition are formed around the test substance-containing wells to assess how well the substances can stop bacteria from growing. The test substances were compared with standard tetracycline, a broad-spectrum antibiotic.

TABLE 4: DIAMETER OF ZONES OF INHIBITION (MM) OF HERBAL TABLETS AGAINST MICROORGANISM

Organism (Bacteria)	F1 Zone of inhibition (mm)	F2 Zone of inhibition (mm)	F3 Zone of inhibition (mm)	Standard Teracycline Zone of inhibition (mm)
S typhi	27	25	25	30
S. aureus	25	20	25	30
E coli	24	24	23	25

FIG. 6: DISC DIFFUSION AGAR PLATE FOR *S. TYPHI, S. AUREUS AND E. COLI*

The results showed that all the test substances exhibited antibacterial activity against the three bacteria tested. Among them, F1 had the highest activity, followed by F3, F2. F1 showed comparable activity to standard tetracycline against *S. typhi and S. aureus*, and slightly lower activity against *E. coli*. F3 showed similar activity to standard tetracycline against *S. aureus*, and slightly lower activity against *S. typhi* and *E. coli*. F2 showed moderate activity, but lower than standard tetracycline against all bacteria.

These findings suggest that *Persea americana* seed extract has potent antibacterial effects and may be useful for the treatment of bacterial infections. IIesanmi *et. al.* also reported avocado oil extract to be active against both gram positive and gram-negative bacteria.

This study holds great significance as it could lead to the development of food preservatives or pharmaceutical compounds that effectively combat food rotting and multidrug resistant microbes [24].

Antifungal Activity: Using the agar well diffusion method, the antifungal activity of herbal tablets containing *Persea americana* seed extract (F1, F2, F3) was assessed. This method measures the ability of the test substances to inhibit the growth of fungi by creating zones of inhibition around the wells containing the substances. The test substances were compared with standard amphotericin B, a potent antifungal drug.

FIG. 7: ANTIFUNGAL ACTIVITY OF HERBAL FORMULATIONS ON *C. ALBICANS*

TABLE 5: DIAMETER OF ZONES OF INHIBITION (MM) OF HERBAL TABLETS AGAINST FUNGAL STRAIN

Organism	F1 Zone of inhibition (mm)	F2 Zone of inhibition (mm)	F3 Zone of inhibition (mm)	Standard Amphotericin Zone of inhibition (mm)
Candida albicans	25	22	20	20

The results showed that all the test substances exhibited antifungal activity against *Candida albicans*, a common cause of fungal infections in humans. Among them, F1 had the highest activity, followed by F2, F3, and standard amphotericin B. F1 had noticeably more activity than amphotericin B standard. While F2 exhibited marginally greater activity than conventional amphotericin B, there was no statistically significant difference between the two. Similar activity was shown by F3 and conventional amphotericin B. These results imply that the seed extract of *Persea americana* possesses strong antifungal properties and could be helpful in the management of fungal infections. Carvajal-Zarrabal*et. al.* reported toxic effect towards fungus *Candida sp.*and *Cryptococcus noformans* with the seed extract of avocado [23].

Cell Viability by MTT Assay: The MTT assay counts the amount of viable cells in a culture using a colorimetric approach. It depends on mitochondrial dehydrogenases reducing MTT intracellularly to formazan. In this study, MTT assay was performed to analyze the toxicity of herbal tablets (F1, F2, F3) of *Persea americana* seed extract and nanoparticles of *Persea americana* seed extract on fibroblast cells isolated from chicken embryo. The result was confirmed by color change as shown below in a 96-well plate and the absorbance read by an ELISA microplate reader.

TABLE 6: PERCENTAGE OF VIABLE CELL

Test substance	Cell viability %
control	87.71
Nanoparticle	60.31
F1	77.25
F2	63.13
F3	45.76

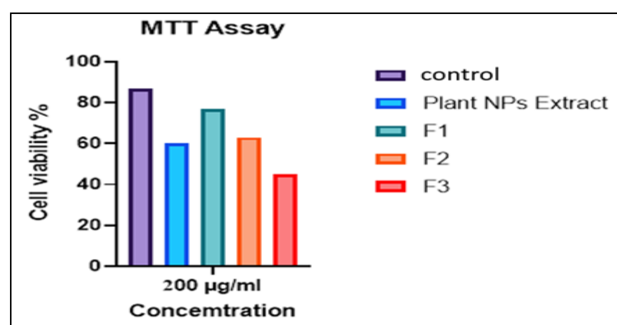

FIG. 8: GRAPH SHOWING % CELL VIABILITY

The findings demonstrated that the test chemical substances had a dose-dependent effect on fibroblast cell viability. F3 had the highest toxicity, followed by nanoparticle, F2, and F1. F1 showed the lowest toxicity and was comparable to the control. These findings suggest that test substances have a significant effect on cell proliferation and may have potential applications in drug screening.

CONCLUSION: The study focused on creating chitosan nanoparticles from avocado seeds and integrating them into herbal tablets to explore their bioactivity. Results highlight these formulations' potential for various therapies. Chitosan nanoparticles, made *via* ionic gelation from avocado seed extracts, were confirmed using UV-Vis and FTIR spectroscopy, validating their formation. Herbal tablets met quality standards in terms of flow, dimensions, weight, hardness, and disintegration time.

These tablets inhibited α-Amylase significantly, particularly F1 and F2, akin to Metformin, hinting at their use as natural diabetes inhibitors with fewer side effects. Additionally, the formulations and nanoparticles displayed potent antioxidant effects, especially those rich in avocado seed extract, offering promise against oxidative stress. They also exhibited concentration-based anti-inflammatory effects, particularly F1, suggesting potential for treating inflammation and warranting further study.

Moreover, these tablets showed significant antibacterial and antifungal properties against various pathogens, indicating their potential for fighting infections. While some formulations like F3 and nanoparticles reduced cell viability dose-dependently, Due of its low toxicity, F1 is a safer option for additional medication development.

The findings suggest that formulations containing avocado seed extract have a variety of bioactivities that make them promising options for treating infections, managing diabetes, antioxidant therapy, and reducing inflammation. However, deeper studies are necessary to understand their mechanisms, components, pharmacokinetics, and

clinical effectiveness before considering them for therapeutic use. These formulations, with their natural origin and multiple effects, hold promise for safer and more effective therapeutic interventions, pending further investigation into their full potential and safety for clinical applications.

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