STM JOURNALS

Journal of
Polymer & Composites

ISSN: 2321-2810 (Online)
ISSN: 2321-8525 (Print)
Volume 14, Issue 5, 2026
September–October
DOI (Journal): 10.37591/JoPC

https://journals.stmjournals.com/jopc

Research JoPC

Synthesis, Characterization, and Biomedical Applications of a Silver Nanoparticle-Coated Chitosan–Fucoidan–Polymer Nanocomposite Loaded with *Persea americana* Leaf Extract

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Abstract

Consuming a natural composite of Persea americana *(Avocado) leaf extract and a chitosan and fucoidan form a biodegradable biopolymer matrix–polymer nanocomposite with biomedical applications, this study explored a potential therapeutic method. GC–MS and UV spectroscopy were used to describe the aqueous leaf extract, which was verified by phytochemical screening to contain flavonoids, tannins, phenols, terpenoids, and alkaloids. The chitosan–fucoidan biodegradable polymer matrix served as a biocompatible stabilizing and encapsulating carrier for silver nanoparticles (AgNPs), enhancing nanoparticle stability, controlled phytochemical release, and overall biomedical performance. To improve bioavailability and activity, it was added to a biopolymer matrix that had been coated with silver nanocomposite. Strong dose-dependent radical scavenging effects were demonstrated by antioxidant capacity as assessed by phosphomolybdic acid, hydrogen peroxide, DPPH, and FRAP assays. Tests of antifungal activity were conducted against* Candida tropicalis *and* Candida albicans*. Pancreatic lipase and α-amylase inhibition assays were used to measure anti-obesity and anti-diabetic potential. Significant protein stabilization and denaturation inhibition were found when anti-inflammatory activity was assessed using heat-induced hemolysis and albumin denaturation assays. In the absence of additional clinical validation, this biopolymer–nanoparticle delivery system shows promise as a treatment candidate for PCOS by providing a biocompatible, environmentally benign method of controlling oxidative stress and inflammation.*

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Received Date: July 21, 2026
Accepted Date: July 29, 2026
Published Date: August 13, 2026

Citation: S. Divya Priya, K. Rajathi, M. E. Pavithra, Mekala Moorthy, G. Nivetha, J. Srinaya. Synthesis, Characterization, and Biomedical Applications of a Silver Nanoparticle-Coated Chitosan–Fucoidan–Polymer Nanocomposite Loaded with *Persea americana* Leaf Extract. Journal of Polymer & Composites. 2026; 14(5): 130–148p.

Keywords: Green synthesis, chitosan–fucoidan, biomedical polymer nanocomposite, antioxidant, antifungal, anti-inflammatory

INTRODUCTION

Lively to 15% to 20% of women in their reproductive years may have PCOS, the most common hormonal condition, depending on the population being studied and the diagnostic criteria [1]. Although the precise pathophysiology of this varied disease is unknown, it is believed to be caused by a complex interaction of genetic, metabolic, and environmental factors. Ovulatory failure, hyperandrogenism, and polycystic ovarian morphology are its distinguishing characteristics [2]. PCOS is associated with negative clinical outcomes, including irregular menstruation and infertility and each of these has been found to be a factor in the deterioration of life quality [3]. In the

first few years following menarche, up to 85% of adolescent menstrual cycles are anovulatory, and under physiological circumstances, up to 59% continue to be anovulatory for up to three years. Furthermore, only 40% of teenagers with irregular periods have polycystic ovaries on ultrasonography [4]. However, other researchers found that the distinctive polycystic ovaries were highly prevalent in adolescent females who were otherwise asymptomatic, suggesting that this condition is frequent in this age group. Moreover, hyperandrogenism seems to be the most important factor in adolescent diagnosis. These results led to the proposal that to diagnose PCOS in teenagers, irregular menstrual cycles must persist for at least two years after menarche and all three criteria must be satisfied [5].

PCOS often develops during puberty, resulting in irregular menstruation, dermatological symptoms (hirsutism, acne, and alopecia), and biochemical changes associated with elevated levels of testosterone, DHEA, androstenedione, and luteinizing hormone (LH), as well as an elevated LH/follicle-stimulating hormone (FSH) ratio and a concurrent decrease in sex hormone binding globulin (SHBG) and insulin-like growth factor (IGF) binding protein [6].

Recent studies suggest that the local inflammatory response of the ovarian theca cells to reactive oxygen species or to cytokines and chemokines produced by dysfunctional adipose tissue may be the cause of hyperandrogenism [7]. It is still unknown how symptoms – like anovulation, hyperandrogenism, and insulin resistance – develop in women with PCOS. Anovulation and hyperandrogenism are the only characteristics of PCOS that have improved in cross-sectional research, in contrast to other symptoms [8].

Persea americana, or avocado, leaves have recently become more well-known due to scientific research that validates their traditional medicinal usage [9]. The aqueous extract of *P. americana* has been reported to exert antihypertensive and vasorelaxant effects in rat aortic rings. The metabolic investigation of *P. americana* aqueous leaf extract in a rat model revealed the presence of phenolic acids, which were products of the breakdown of flavonols by the intestinal microbiota [10]. The leaves are claimed to be an effective antitussive, antidiabetic and arthritic pain reliever by the traditional medicine practitioners of the Ibibio tribe in South Nigeria. The analgesic and anti-inflammatory properties of the leaves have been reported [11].

Natural polymers – such as chitosan and fucoidan – have gained significant attention in biomedical research due to their biodegradability, biocompatibility and non-toxic nature [12]. The combination of these two materials results in a functional biodegradable polymer composite as an efficient carrier and a stabilizing matrix for silver nanoparticles to improve stability, control the release and bioactivity of the nanoparticles [13]. These polymer nanocomposites have great potential for applications in drug delivery, wound healing, tissue engineering and treatment of disorders related to oxidative stress and inflammation, making them attractive materials for advanced biomedical applications [14].

Chitosan is a product of deacetylation of chitin, in which a portion of the N-acetylglucosamine moieties are converted to glucosamine units [15]. The chitosan structure has many protonated $–NH_2$ groups, which make it soluble in acidic aqueous solutions. Its pKa value is about 6.5. Chitosan is soluble when about half of all amino groups are protonated [16]. This stage is usually finished with alkaline treatments like NaOH [17]. Chitosan may convert glucosamine units ($–NH_2$) into the soluble protonated form ($–NH^{+3}$) in diluted aqueous acidic solutions below its pKa (~6.3), despite being a weak base and insoluble in water. The molecular weight, level of acetylation, and biological origin of chitosan all affect its solubility [18, 19].

Brown seaweed and certain marine animals (such sea urchins and sea cucumbers) contain fucoidans, which are polysaccharides with significant amounts of L-fucose and sulfate ester groups [20]. Due to their diverse biological activities, which include anticoagulant and antithrombotic, antiviral, antitumor and immunomodulatory, anti-inflammatory, blood lipid reduction, antioxidant and anticomplementary properties, activity against hepatopathy, uropathy, and nephropathy, gastric protective effects, and

therapeutic potential in surgery [21], fucoidans isolated from various species have been thoroughly studied for the past ten years [22]. Since fucoidans are more readily available from a variety of inexpensive sources than other sulfated polysaccharides, an increasing number of fucoidans have been studied in recent years to create medications or functional foods [23]. The structure and bioactivity of fucoidans isolated from brown seaweed, as well as the connections between their structures and bioactivity, are summarized in this work [24].

Therefore, the present study aimed to develop a biodegradable chitosan–fucoidan–polymer nanocomposite as a biocompatible carrier and stabilizing matrix for silver nanoparticles (AgNPs) loaded with *Persea americana* leaf extract as shown in Figure 1. The synthesized polymer nanocomposite was characterized and evaluated for its antioxidant, antifungal, anti-obesity, antidiabetic, and anti-inflammatory activities to explore its potential biomedical applications in the management of PCOS.

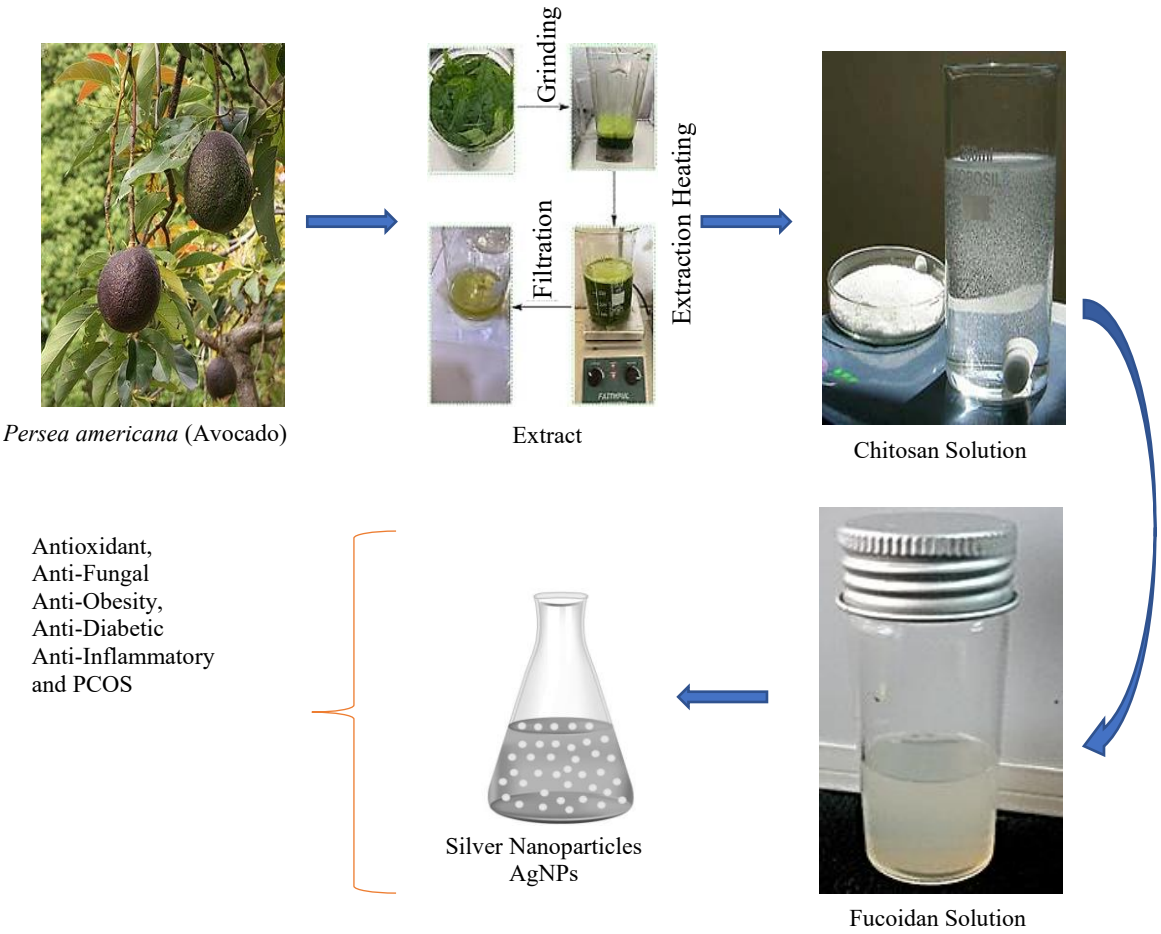

Figure 1. Therapeutic approach using a natural biopolymer composite of *Persea americana* (avocado) leaf extract combined with a fucoidan–chitosan–silver nanoparticle complex.

MATERIALS AND METHODS

Sample Collection & Identification

The collection and proper identification of plant material are important to ensure the authenticity and quality of the study. *Persea americana* (avocado) leaves were selected due to their rich phytochemical composition and potential biological activities. The collected leaves were authenticated and processed for extract preparation and further experimental analysis.

Preparation of Plant Extract

Avocado leaves were collected from a nearby area in Coimbatore, Tamil Nadu. The avocado leaves

were dried for about 10 days at room temperature. Then the sample was dried and powdered. Dried leaves were taken and blended into a fine powder. 20 g of the powder was weighed, mixed into 200 ml of distilled water, kept in a heating mantle and vaporized until the dry extract was collected.

Synthesis and Preparation of Plant Extract Along with Chitosan–Fucoidan Biopolymer Composite-Coated with Silver Nanoparticles

100 milliliters of water were used to dissolve 0.33 grams of silver nitrate. The solution was combined with 1 g of extract. The silver nitrate solution was mixed with one milliliter of fucoidan solution and one milliliter of chitosan solution, heated to 90 degrees Celsius on a hot plate, and constantly swirled with a magnetic stirrer for one to two hours. Following the stirring procedure, the mixture was put into 2 ml centrifuge tubes and spun for roughly 10 minutes at 10,000 rpm. After that, the tubes were cleaned and centrifuged once more for roughly ten minutes. This procedure was repeated two or three times to ensure that the sample was extracted completely (Figure 1).

Role of Chitosan and Fucoidan in the Polymer Nanocomposite

The composite was predominantly based on the chitosan biodegradable polymer matrix, which offered a biocompatible matrix for encapsulating *Persea americana* leaf extract and homogeneously distributing silver nanoparticles (AgNPs**)** (Figure 2). The amino groups of chitosan contributed to the binding and stabilization of AgNPs and to the enhancement of mechanical stability and controlled release of the bioactive compounds [25]. Fucoidan acted as a natural stabilizing and biointeractive polysaccharide by preventing nanoparticle aggregation, enhancing colloidal stability, and promoting biological interactions through its sulfated functional groups [26]. The synergistic combination of chitosan and fucoidan produced a stable biodegradable polymer nanocomposite with enhanced antioxidant, antimicrobial, and anti-inflammatory properties, making it suitable for biomedical applications [27].

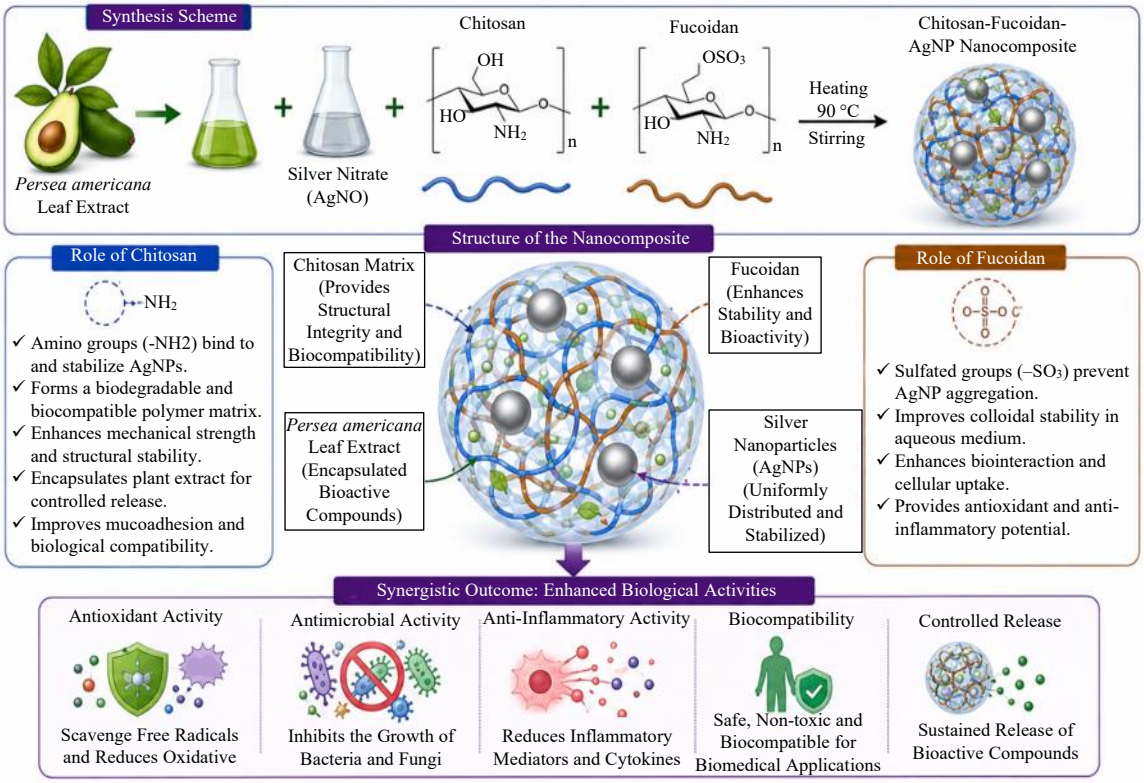

Figure 2. Schematic representation of the individual and synergistic roles of chitosan and fucoidan in the *Persea Americana* leaf extract-loaded silver nanoparticle (AgNP)-coated polymer nanocomposite.

Phytochemical Qualitative Screening of *Persea Americana* Leaf Extract

The usual protocols were followed when conducting the phytochemical screening test for the presence of phytoconstituents. Compared to fruit or seed, avocado leaves have a greater concentration of beneficial plant compounds such flavonoids and phenols. Antioxidants called flavonoids have anti-inflammatory, anti-clotting, anti-diabetic, and anti-cancer properties. They also shield our nerve cells from deterioration. Antioxidants called phenols shield biomolecules – like proteins, lipids, and DNA – from oxidative damage. Chronic conditions including cancer and cardiovascular illnesses are influenced by oxidative damage. Plant phenols can lower the risk of cancer by interfering with the cancer process.

Characterization of *Persea Americana* Leaf Extract-Coated with Silver Nanoparticles from the Chitosan–Fucoidan Biopolymer Composite

Characterization of the synthesized nanocomposite is essential to determine its chemical composition and confirm the presence of bioactive compounds. In the present study, Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed to identify the phytochemical constituents present in the *Persea americana* leaf extract-coated silver nanoparticles synthesized using the chitosan–fucoidan biopolymer composite.

Gas Chromatography–Mass (GC–MS) Spectrometric Analysis

The GC–MS is a fantastic technology that has grown in popularity because of its selectivity and sensitivity through the removal of template ions and selective reaction monitoring as compared to conventional methods (TLC, HPTLC, and HPLC). When a strong matrix environment is available, the reaction monitoring described above can detect even the smallest levels of phytoconstituents with high accuracy. The generated mass spectra were analyzed to determine the embedded constituents using the internal references mass spectra from NIST. EOLE found 26 plant components in addition to the retention time (RT), molecular formula, molecular weight (MW), and percentage (%) peak area stated [28].

Anti-Oxidant Activity

Evaluation of antioxidant activity is important to determine the ability of the synthesized nanocomposite to scavenge free radicals and reduce oxidative stress. In the present study, the antioxidant potential of the *Persea americana* leaf extract-coated silver nanoparticles synthesized using the chitosan–fucoidan biopolymer composite was assessed using DPPH, FRAP, hydrogen peroxide scavenging, and phosphomolybdate assays.

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) Assay in Microplate Assay

The DPPH assay relies on antioxidant chemicals reducing the DPPH radical, a persistent free radical that absorbs at 517 nm and has a deep violet color. DPPH is converted to DPPH-H when antioxidants give it hydrogen atoms or electrons, giving it a yellowish hue. The sample's antioxidant activity is directly correlated with the degree of decolorization.

FRAP (Ferric Reducing Antioxidant Power) in Microplate Assay

To use the FRAP microplate assay to measure test samples' capacity to convert ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions to assess their antioxidant capability. The FRAP test relies on antioxidants' capacity to lower the Fe^{3+}-TPTZ (2, 4, 6tripyridyl-s-triazine) complex to the blue Fe^{2+}-TPTZ complex, which absorbs light at 593 nm.

Hydrogen Peroxide Assay (H_2O_2)

One reactive oxygen species (ROS) that can result in oxidative damage is hydrogen peroxide (H_2O_2). By tracking the rise in H_2O_2 concentration, which absorbs light at 240 nm, this assay assesses the capacity of antioxidants to neutralize H_2O_2. The assay provides information on the physiological state of plant tissues by evaluating oxidative stress and metabolic activity.

Phosphomolybdate Assay

The phosphomolybdate assay depends on reducing agents' capacity to interact with phosphomolybdic acid, a heteropoly acid that contains molybdenum in its maximum oxidation state (Mo^{6+}). Usually, a spectrophotometer is used to measure the absorbance of the resultant-colored complex at a wavelength of 600–700 nm. To quantify antioxidant activity in different samples, the results are frequently stated in relation to standard substances such as ascorbic acid or gallic acid.

Antifungal Activity

The antifungal activity of the test sample was evaluated against selected fungal pathogens to determine its inhibitory potential. The efficacy of the sample was assessed by measuring its ability to inhibit fungal growth using the agar well diffusion method.

Agar Well Diffusion Method

A test sample's antifungal activity is assessed using the Agar Well Diffusion Method. The zone of inhibition (ZOI) against particular fungal strains is measured to achieve this. An antifungal agent is released from a well into an agar medium that has already been inoculated with a fungal culture for the procedure to work. The diameter, in millimeters, of the clear zone around the well, where fungal growth has been inhibited, is used to measure the effectiveness of the agent.

Anti-Obesity Activity

The anti-obesity potential of the test sample was evaluated by determining its ability to inhibit pancreatic lipase, a key enzyme involved in the digestion and absorption of dietary fats. Pancreatic lipase inhibition is considered an important strategy for reducing fat absorption and managing obesity.

Pancreatic Lipase Inhibition Assay

The extent to which *Persea americana* (Avocado) leaf extract inhibits pancreatic lipase activity is measured using the lipase assay. This enzyme is crucial for breaking down dietary triglycerides into monoglycerides and fatty acids that the intestines can absorb. Leaf extract is an effective method for managing obesity because it can reduce fat absorption and digestion by blocking this enzyme. This assay assesses the enzyme's activity with a specific substrate like olive oil emulsion.

Anti-Diabetic Activity

The anti-diabetic potential of the test sample was evaluated by determining its ability to inhibit carbohydrate-hydrolyzing enzymes involved in glucose metabolism. Enzyme inhibition assays provide a simple and effective approach for assessing the potential of natural products to reduce postprandial blood glucose levels.

Alpha-Amylase Inhibitory Activity

Starchy foods are broken down in the body by an enzyme called α-amylase, which converts starch into smaller sugars like glucose and maltose. This study investigates how effectively avocado extract can slow down or block this process. To assess this, a special chemical called DNS (3,5-dinitrosalicylic acid) is used, which reacts with the sugars released from the starch and forms a colored solution. The intensity of this color is measured using a spectrophotometer set to 540 nm. A less intense color indicates that the avocado extract has successfully inhibited the enzyme and less sugar was produced.

Anti-Inflammatory Activity

Inflammation is the body's natural response to injury or infection, but excessive inflammation can damage tissues. The anti-inflammatory activity of the test sample was evaluated using the albumin denaturation assay and the heat-induced red blood cell (RBC) membrane stabilization assay.

Albumin Denaturation Assay

The purpose of the egg albumin denaturation test is to ascertain whether a substance can inhibit or reduce the denaturation of egg albumin under certain conditions. Denaturation involves a permanent

alteration of the protein structure, which leads to loss of its biological function. Egg albumin is used as a model protein in this experiment, and it undergoes denaturation by being exposed to highly acidic or basic solutions, elevated temperatures, or other denaturing agents. During the denaturation process, the three-dimensional structure of the protein loses its native shape, which causes changes to its physical and chemical properties.

The ability of the substance to be tested to counteract this process is then measured and analyzed. This assumes that anti-inflammatory compounds can stabilize the protein conformation and preventing its denaturation, which is often associated with inflammatory processes in tissues. Therefore, a compound that significantly reduces or inhibits the acid-induced denaturation of egg albumin in this assay is considered to possess anti-inflammatory activity.

Heat-Induced Hemolysis Assay (RBC)

The assay is based on the principle that increased temperatures lead to the destabilization of the red blood cell (RBC) membrane. The increased permeability of the membrane results in cell swelling and eventual lysis. Hemoglobin present in the cell is released into the surrounding medium upon the rupture of cells. Spectrophotometry can, therefore, be used to determine the amount of released hemoglobin.

The RBC assay involves incubating a suspension of RBCs for a fixed period at varying temperatures, starting from a normal body temperature, i.e., 37°C to as high as 55°C or higher. After the incubation period, the supernatant is separated from the cell pellet by centrifugation. The absorbance of the supernatant at 540 nm is then determined. A higher level of cell lysis will result in higher absorbance at 540 nm. The percentage of RBC lysis is determined by comparing the obtained value to the values obtained from the corresponding positive and negative controls.

RESULT

Phytochemical Qualitative Screening of *Persea Americana* Leaf Extract

The *Persea americana* leaves were subjected to various phytochemical analyses in an aqueous solvent. Table 1 reveals the presence of essential phytoconstituents in the *Persea americana* leaf extract.

Table 1. Phytochemical screening test.

S. N.	Test	Aqueous extract
1.	Alkaloids	++
2.	Flavanoids	+++
3.	Saponins	–
4.	Glycosides	+
5.	Tannins	+
6.	Phenols	+
7.	Proteins	++
8.	Carbohydrates	–
9.	Terpenoids	+
10.	Triterpenoids	+
11.	Phytosterols	+
12.	Polyphenols	+
13.	Anthraquinones	–
14.	Quinones	–
15.	Steroids	+

Characterization of Silver Nanoparticles from the Polymer Matrix

The synthesized *Persea americana* leaf extract-loaded chitosan–fucoidan biopolymer composite coated silver nanoparticles were characterized to identify the bioactive compounds associated with the polymer matrix. Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed to determine the phytochemical constituents present, which may contribute to the synthesis, stabilization, and biological activities of the nanoparticles.

Gas Chromatography–Mass (GC–MS) Spectrometric Analysis

The *Persea americana* leaf extract in water was analyzed using gas chromatography–mass spectrometry (GC–MS). The GC–MS-identified bioactive compounds are listed in Table 2. Several biochemical substances, including quinine, phenol, and alkanes, were found in the study conducted to determine the type of compounds contained in the leaf extract GC–MS analysis has been represented in Figure 3. The abundance of the substances that showed up in the GC–MS chromatography was used to infer their presence.

Table 2. Bioactive compounds present in aqueous extract of *P. americana* leaf, chitosan–fucoidan biopolymer composite-coated AgNPs.

S. N.	RT(m)	Name of the compound	Molecular – formula	Molecular – weight	Peak – area (%)
01	9.619	Dodecane,2, 6, 11-trimethyl	$C_{15}H_{32}$	212.41	7.83
02	9.708	Phenol 2, 4 bis (1, 1-dimethyl ethyl)	$C_{14}H_{22}O$	206.32	10.33
03	17.252	Heptacosane	$C_{27}H_{56}$	380.75	14.82
04	7.464	Thymoquinone	$C_{10}H_{12}O_2$	164.20	20.02
05	15.141	Eicosane	$C_{20}H_{42}$	282.55	22.42
06	16.229	Octacosane	$C_{28}H_{58}$	394.77	24.15
07	15.319	Heneicosane	$C_{12}H_{44}$	296.58	26.04
08	6.953	Estragole	$C_{10}H_{12}O$	148.20	36.96
09	7.787	Thymol	$C_{10}H_{14}O$	150.22	100.00

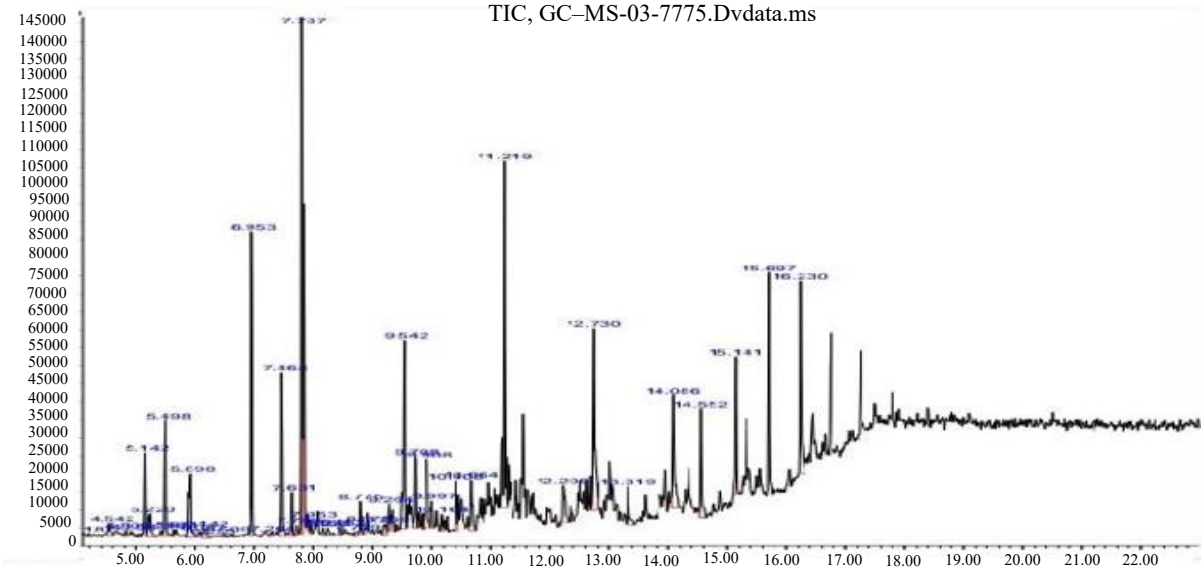

Figure 3. Bioactive compounds present in aqueous extract of *Americana* leaf extract, chitosan–fucoidan biopolymer composite complex-coated AgNPs.

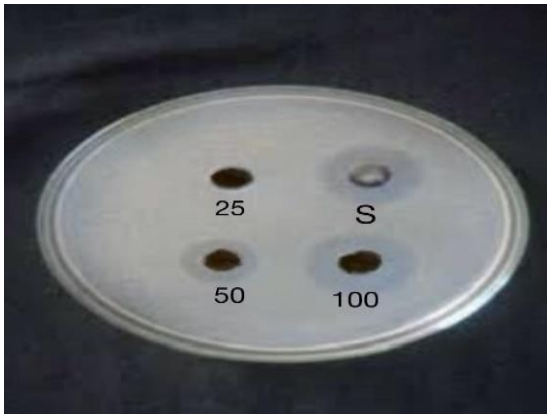

Figure 4. *Candida albicans*.

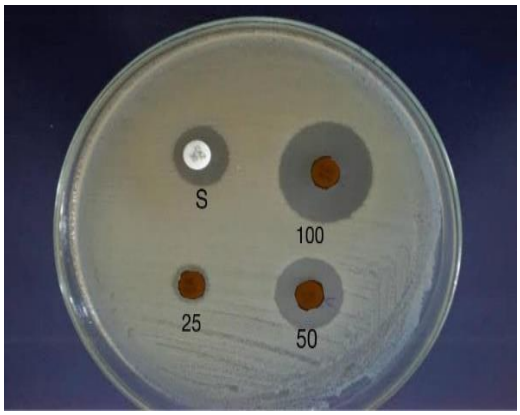

Figure 5. *Candida tropicalis.*

Antifungal Activity

The antifungal activity against the tested fungal strains is presented in Table 3. The antifungal activity of chitosan–fucoidan-coated Ag was analyzed against fungal strains *Candida tropicalis*, and *Candida albicans*. Figures 4 and 5 represent the antifungal activity of chitosan–fucoidan-coated AgNPs of *Persea Americana* leaf extract for various bacteria in disc diffusion assay. Results showed that Chitosan–fucoidan-coated AgNPs demonstrated excellent antifungal activity against a range of fungi. *Candida tropicalis* exhibited the zone measuring 5 mm and *C. albicans* exhibited the zone measuring 9 mm as shown in Table 3. The diameter of the inhibition zone reflects the magnitude of the susceptibility of microbes. The strains susceptible to chitosan–fucoidan-coated AgNPs exhibited a larger zone of inhibition, whereas resistant strains exhibit a smaller zone of inhibition. According to the zone of inhibition, *A.* C.albicans *flavus* exhibited the highest sensitivity toward Chitosan–fucoidan-coated AgNPs while C.tropicalis *albicans* showed the least sensitivity among the tested microbes.

Table 3. In-vitro antifungal activity of *P. americana* leaf extract – chitosan–fucoidan-coated polymer nanocomposite.

Samples	Concentrations (µg/ml)	Organisms/zone of inhibition (mm)	
		Candida albicans	***Candida tropicalis***
Samples	25	2	1
	50	5	2
	100	9	5
Standard (Amoxicillin)	10 µl/disc	4	1

Anti-Oxidant Activity

The antioxidant efficacy of silver nanoparticles (AgNPs) was assessed using the DPPH radical scavenging assay to determine the scavenging activity percentage (SA %). The results are presented in Table 4 and demonstrate a concentration-dependent increase in SA%, confirming the antioxidant potential of AgNPs. At 200 ml, the SA% was 54.4%, increasing to 48.8% at 100 ml, 46.4% at 50 ml, 41.2% at 25 ml, and 37.8% at 12.5 ml are shown in Figure 6. The decrease in percentage inhibition of optical density at 517 nm on varying concentrations suggests an impressive activity of the samples tested by enhanced free radical scavenging activity, which was determined to have an IC50 value of 1.86µg/ml. This implies that AgNPs synthesized from *Persea Americana* can be used as antioxidants in biodegradable panty liners as an antioxidant functional material.

Table 4. DPPH assay of AgNPs from *Persea Americana* leaf extract with polymer matrix.

Concentration of extract	% of inhibition
200	54.4
100	48.8
50	46.4
25	41.2
12.5	37.8

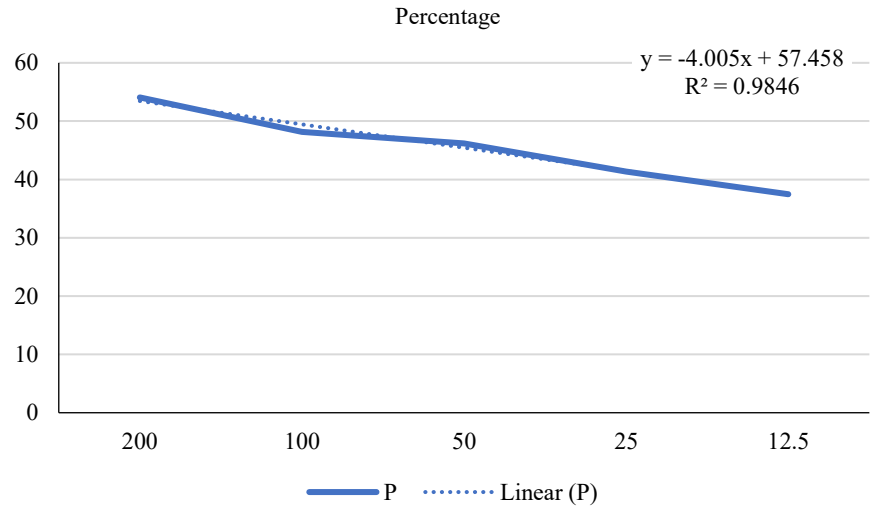

Figure 6. Percentage of DPPH activity of AgNPs from *Persea Americana* leaf extract.
Note: IC50 Value = 1.86 µg/mL**.**

This study evaluates the ferric reducing ability of silver nanoparticles (AgNPs) synthesized from *Persea Americana* and it was used to measure the reducing power of the NPs, indicating their electron-donating ability. The results demonstrated Table 5 and a concentration-dependent decrease in FRAP value, confirming the reducing potential of AgNPs. The FRAP value was 20.72 at 20 mg/ml, 23.41 at 40 mg/ml, 28.26 at 60 mg/ml, 42.34 at 80 mg/ml and reaching 55.26 at 100 mg/ml and IC50 value is 4.82µg/ml as shown in Figure7. The reduction in optical density (OD) at 620 nm with increasing concentration indicates enhanced ferric ion reduction, highlighting the strong reducing capability of Extracts.

Table 5. FRAP assay of AgNPs from *Persea Americana* leaf extract coated with polymer matrix.

Concentration of extract	**% of inhibition**
20	20.72
40	23.41
60	28.26
80	42.34
100	55.26

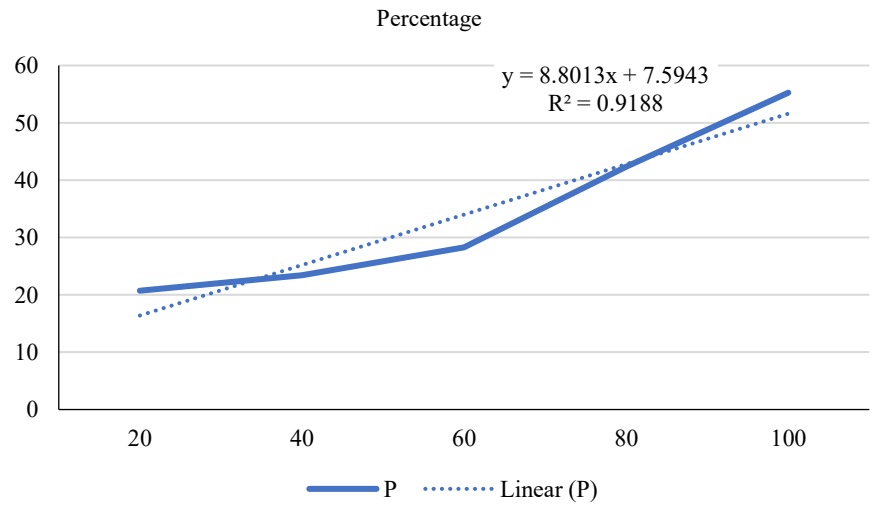

Figure 7. Percentage of FRAP assay of AgNPs from *Persea americana* leaf extract with polymer matrix.
Note: IC50 Value = 4.82 µg/mL.

This study evaluates the hydrogen peroxide scavenging ability of silver nanoparticles (AgNPs) and the assay was performed to assess the scavenging activity percentage (SA %) of AgNPs, indicating their ability to neutralize hydrogen peroxide. The results demonstrated a concentration-dependent increase in SA%, confirming the antioxidant potential of AgNPs. At 20 ml, the SA% was 11.125%, increasing to 17.625% at 40 ml, 21.25% at 60 ml, 39.625% at 80 ml, and reaching 59.375% at 100 ml and IC50 value 4.76 µg/ ml as shown in Table 6 and Figure 8. The reduction in optical density (OD) at 240 nm with increasing concentration indicates enhanced hydrogen peroxide neutralization, highlighting the strong scavenging ability of AgNPs.

Table 6. H_2O_2 assay of AgNPs of *Persea american a* leaf extract coated with complex.

Concentration of extract	% of inhibition
20	11.125
40	17.625
60	21.25
80	39.625
100	59.375

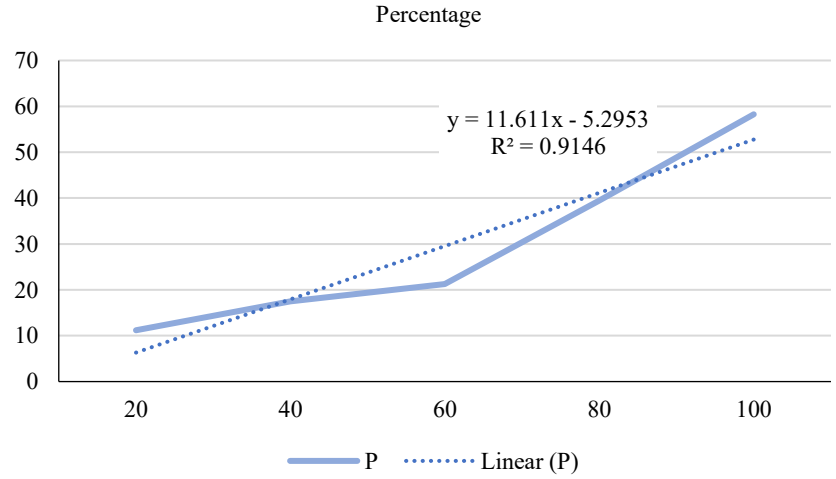

Figure 8. Percentage of H_2O_2 assay of AgNPs from *Persea americana* leaf extract with polymer matrix.
Note: IC50 Value = 4.76 µg/ml.

The antioxidant efficacy of silver nanoparticles (AgNPs) was assessed using the DPPH radical scavenging assay to determine the scavenging activity percentage (SA %) are summarized in Table 7. The results conformed a concentration-dependent increase in SA%, confirming the antioxidant potential of AgNPs. At 20 ml, the SA% was 34.23%, increasing to 43.96% at 40 ml, 36.03% at 60 ml, 47.06% at 80 ml, and 56.23% at 100 ml as shown in Figure 9. The reduction in optical density (OD) at 517 nm with increasing concentration indicates enhanced free radical scavenging activity, and IC50 value is 4.38 µg/ml highlighting the potent antioxidant nature of AgNPs. These findings suggest that AgNPs synthesized from *Persea Americana leaf extract coated with complex* exhibit significant antioxidant properties, making them suitable for applications in biodegradable panty liners.

Table 7. Phosphomolybdate assay of AgNPs of *Persea americana* leaf extract coated with polymer matrix.

Concentration of extract	% of inhibition
20	34.23
40	43.96
60	36.03
80	47.06
100	56.23

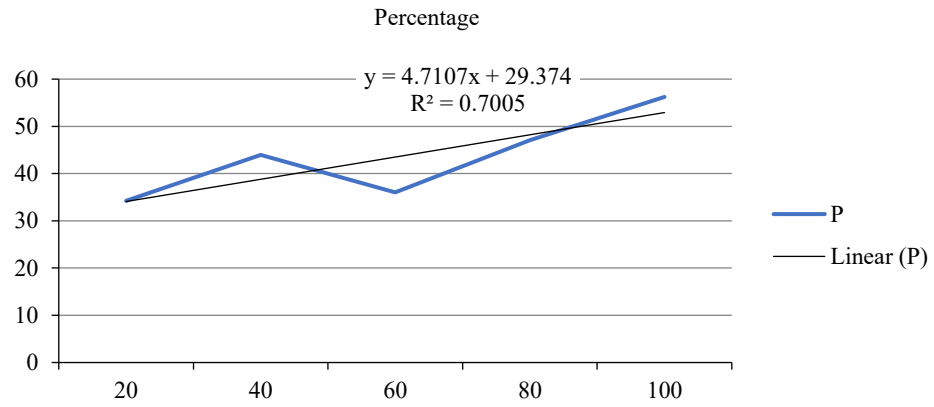

Figure 9. Percentage of phosphomolybdic activity of AgNPs from *Persea americana* leaf extract with coated polymer matrix.
Note: IC50 Value = 4.38 µg/mL.

Anti-Obesity Activity

The in vitro anti-obesity assay of *Persea americana* aqueous extract showed a concentration-dependent increase in percentage inhibition of pancreatic accumulation. At the lowest concentration (25 µl), the inhibition was minimal (5.37%), while the highest inhibition (86.40%) was observed at 1000 µl as represented in Table 8 & Figure 10. This indicates that the extract has significant lipase inhibitory activity, suggesting its potential in managing obesity through natural means.

Table 8. Anti-obesity activity of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.

Concentration of extract	% of inhibition
25µl	5.37
50µl	14.74
75µl	21.92
100µl	37.18
250µl	53.11
500µl	64.71
750µl	79.98
1000µl	86.40

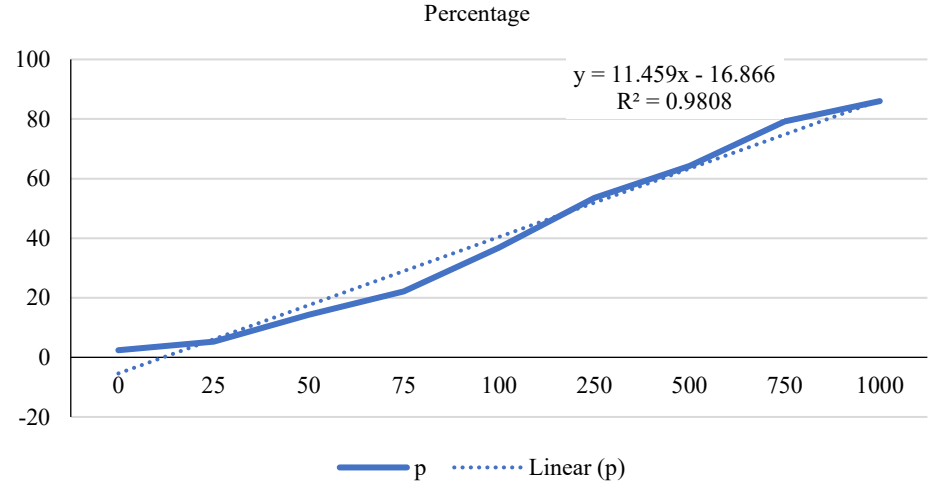

Figure 10. Anti-obesity of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.
Note: IC50 Value = 5.84 µg/mL.

Anti-Diabetic Activity

The antidiabetic potential was evaluated by measuring the percentage inhibition at various concentrations (0–1000 µL) of the synthesized fucoidan and chitosan-coated silver nanoparticles (AgNPs). The inhibition values showed a concentration-dependent increase, indicating that higher concentrations of nanoparticles led to stronger antidiabetic effects as shown in Table 9 and Figure 11.

Table 9. Anti-diabetic activity of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.

Concentration of the extract	% of inhibition
0 µl	2.65
25 µl	10.27
50 µl	12.47
75 µl	25.18
100 µl	34.62
250 µl	42.81
500 µl	57.62
750 µl	63.46
1000 µl	72.56

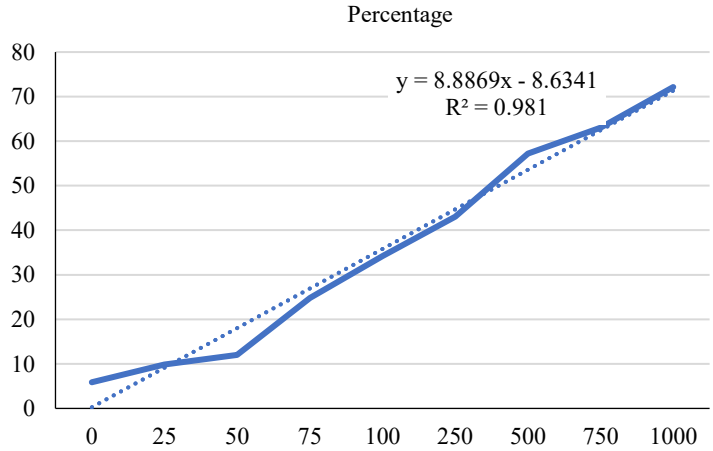

Figure 11. Anti-diabetic of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.
Note: IC50 Value = 6.60 µg/mL.

Anti-Inflammatory Activity

The in-vitro egg albumin denaturation assay was used to assess the anti-inflammatory activity of *Persea americana* with AgNps-coated polymer matrix are presented in Table 10. The aqueous extract of *Persea americana* leaves-coated complexes showed the most significant inhibition of protein denaturation among all the extracts tested as illustrate in Figure 12. This finding suggests that the extract has the potential to be a natural anti-inflammatory agent, which could be particularly useful for managing inflammation related to polycystic ovary syndrome (PCOS).

Table 10. Albumin denaturation assay of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.

Concentration of the extract	% of inhibition
0 µl	1.92
25 µl	7.17
50 µl	11.36
75 µl	16.56
100 µl	32.37
250 µl	80.58
500 µl	83.23
750 µl	85.45
1000 µl	94.87

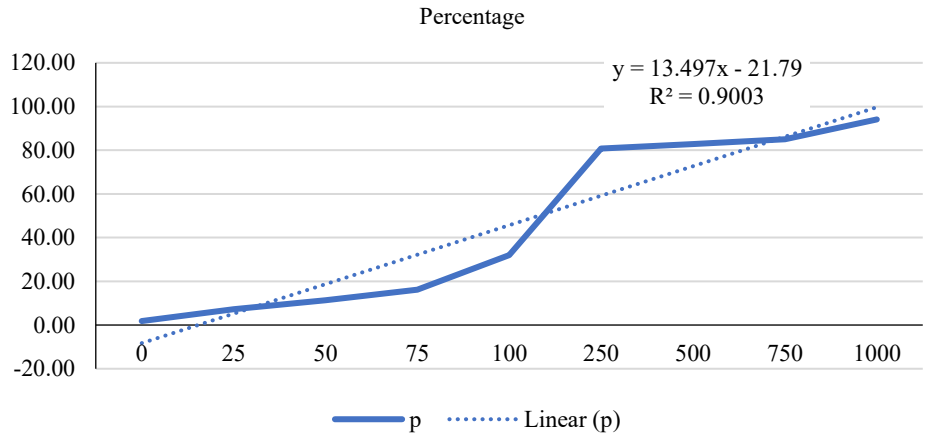

Figure 12. Albumin denaturation assays of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.
Note: IC50 Value = 5.32 µg/mL.

The heat-induced haemolysis assay revealed the membrane stabilizing effect of the extract, thus contributing to the anti-inflammatory potential of the extracts as shown in Table 11 and Figure 13. This implies that the extracts might protect cell membranes against damage, hence suppressing the inflammatory responses.

Table 11. RBC assay of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.

Concentration of the extract	% of inhibition
0 µl	0.85
25 µl	3.36
50 µl	7.47
75 µl	31.32
100 µl	49.48
250 µl	65.19
500 µl	75.22
750 µl	78.26
1000 µl	80.93

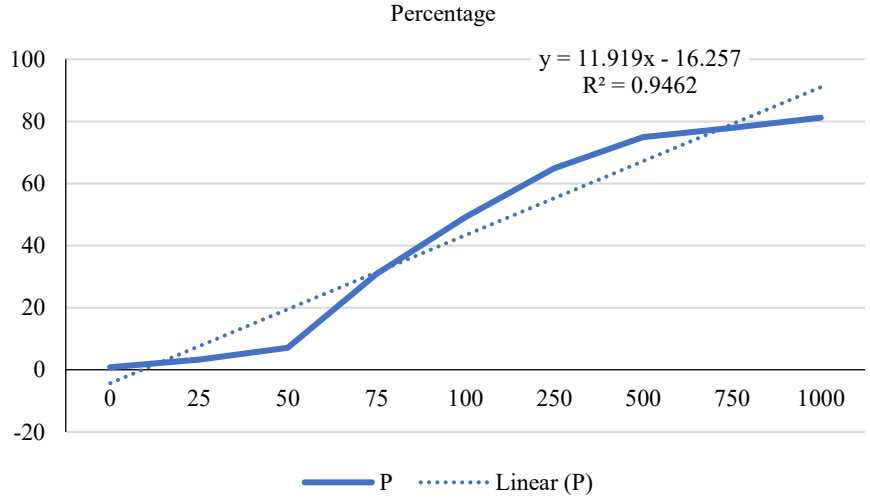

Figure 13. RBC assay of the aqueous extract of *Persea americana* with AgNPs-coated polymer matrix.
Note: IC50 Value = 5.56 µg/mL.

DISCUSSION

Our study results suggest that the *Persea americana* extract and chitosan and fucoidan act as a polymeric matrix that stabilizes silver nanoparticles and supports functional performance. Significant antifungal, antioxidant, anti-obesity, anti-diabetic, and anti-inflammatory qualities were demonstrated by the extract, highlighting its significance in natural treatments and medical research.

Role of the Chitosan–Fucoidan–Polymer Matrix in Nanocomposite Stabilization

Although detailed physicochemical characterization techniques – such as FTIR, SEM, and XRD – were not performed in the present study, the chitosan–fucoidan biodegradable polymer matrix is expected to play an important role in stabilizing and dispersing the silver nanoparticles. Chitosan provides a biocompatible polymeric network through its amino groups, while fucoidan, owing to its sulfated polysaccharide structure, contributes to nanoparticle stabilization and minimizes aggregation [29]. This biodegradable polymer matrix is, therefore, expected to improve the dispersion of AgNPs and facilitate the incorporation of *Persea americana* leaf phytochemicals within the nanocomposite.

Qualitative Phytochemical Tests

An aqueous extract from *Persea americana* leaves was subjected to phytochemical screening, which identified several bioactive chemicals that may have therapeutic value. Flavonoids and alkaloids, along with other components such proteins, phenols, glycosides, tannins, terpenoids, triterpenoids, phytosterols, polyphenols, and steroids, were found to be strongly present, as Table 1 illustrates. Nevertheless, substances including quinones, anthraquinones, polysaccharides, and saponins were absent.

Strong antibacterial, anti-inflammatory, and antioxidant qualities are indicated by the notable concentrations of flavonoids and alkaloids. The extract's ability to treat microbial infections and oxidative stress is further supported by the presence of phenolic compounds, tannins, and glycosides. Its pharmacological profile is further improved by the identification of terpenoids and triterpenoids, which support its anti-inflammatory and anticancer properties. These findings validate the use of *P. americana* leaves in the synthesis of chitosan–fucoidan biopolymer composite-coated with silver nanoparticles and the development of natural treatments by confirming that they are a rich source of secondary metabolites.

GC–MS Analysis

The aqueous extracts of the leaves of *Persea americana* were found to contain several bioactive compounds, many of which are known to be beneficial when present in medicinal plants, according to the GC–MS results. Table 2 and Figure 3 below show the nine major constituents that were identified based on their percentage peak area, molecular formulae, and retention time. The most common component was thymol, a monoterpenoid phenol with antibacterial and antioxidant properties, which constituted 100% of the peak area. Heneicosane (26.04%), a long-chain hydrocarbon with possible antibacterial qualities, and estragole (36.96%), a substance with antifungal and anti-inflammatory characteristics, were two other important components. Among the alkanes, eicosane, octacosane, and heptacosane stood out since they are frequently linked to surface-active and antibacterial qualities. Commonly present in medicinal plants, thymoquinone (20.02%) is known for its strong anti-inflammatory and antioxidant properties. The bioactivity of the extract is further supported by the presence of hydrocarbons and phenol derivatives. These results suggest that the various bioactive phytochemicals present in the aqueous extract of *P. americana* leaves could be responsible for the reported antifungal, antioxidant, and anti-obesity activities of the chitosan–fucoidan-coated AgNPs.

Antifungal Activity

Chitosan–fucoidan-coated silver nanoparticles (AgNPs) produced using *Persea Americana* leaf extract have been examined for their efficacy in vitro against *Candida albicans* and *Candida tropicalis* through a disc diffusion test. The antifungal activity of the nanoparticles was found to be dose-dependent. The zone of inhibition reached 9 mm for *Candida albicans* and 5 mm for *Candida tropicalis* at the maximum tested concentration of 100 µg/ml, both of which were greater than those seen with the

common antifungal drug amoxicillin (4 mm and 1 mm, respectively). These findings show that chitosan–fucoidan-coated AgNPs have better antifungal potential than traditional antibiotics. The inhibitory zones show that *C. albicans* was more susceptible to the nanoparticles than *C. tropicalis*, indicating species-specific changes in susceptibility that may be brought about by variations in resistance mechanisms or cell wall composition. Overall, the results support the possible use of biogenic AgNPs in the fight against fungal diseases and show their promising role as strong antifungal agents, particularly at higher doses.

Anti-Oxidant Activity

It is thought that the antioxidants' capacity to donate hydrogen is connected to their impact on the DPPH, FRAP, H_2O_2, and phosphomolybdate assays. To prevent the harmful effects of free radicals in several conditions, such as cancer and PCOS diseases, scavenging actions for free radicals are essential. An established technique for assessing the antioxidant activity of plant extracts with coated complex is free radical scavenging utilizing assays. According to our findings, the extracts exhibit free radical scavenging activity when compared to normal Trolox, as indicated in Table 4.

Anti-Obesity Activity

The ability of *Persea americana* leaf aqueous extract to inhibit pancreatic lipase, a crucial enzyme involved in the digestion of dietary fat, was used to assess the extract's in vitro anti-obesity effectiveness. To replicate the natural triglyceride breakdown process, the assay used an olive oil emulsion as a substrate. The extract clearly inhibited lipase activity in a concentration-dependent manner, according to the results. Only 5.37% inhibition was seen at the lowest dose (25 µl), suggesting very little enzyme suppression. However, when it came to the highest dilution (1000 µl), the inhibition reached an impressive 86.40%, suggesting that the extract could be a powerful tool in the fight against obesity by suppressing lipid hydrolysis. The study established that the leaves of *P. americana* had the ability to lower the amount of total fat accumulated in the body due to the inhibition of fat absorption. This makes them a valuable source of natural lipid-lowering agents that could be used to produce herbal medicine.

Anti-Diabetic Activity

In our experiment, the ability of the extract to reduce blood sugar levels by inhibiting the breakdown of starch was determined using the α-amylase inhibition assay. The results of the experiment indicate that the extract can reduce the amount of sugar in the blood since the components of the extract can inhibit the action of α-amylase. The results of the experiment corroborate with existing literature that indicates that various extracts of plants possess anti-diabetic properties. From the experiment, it can be concluded that the extract of *Persea Americana* complex mixture contains compounds that can be used to treat diabetes.

Anti-Inflammatory Activity

Heat-induced hemolysis and albumin denaturation inhibition assays were conducted to evaluate the extract's anti-inflammatory properties. Thus, proving that higher dilutions of the extract provided better protection against albumin denaturation by inhibiting it confirmed the extract's anti-inflammatory potentials. Therefore, the *Persea americana* extract and its coated complexes can be used to manage inflammatory conditions because their anti-inflammatory properties were comparable to those reported for other natural compounds and extracts in earlier studies [30].

Synergistic Contribution of the Polymer Matrix to Biological Activity

The improved organic activities observed in this study may be attributed to the synergistic interaction between the *Persea americana* leaf extract, silver nanoparticles, and the chitosan–fucoidan polymer matrix. Chitosan owns inherent antimicrobial and biocompatible properties, whereas fucoidan exhibits antioxidant and anti-inflammatory activities. Composed, these biodegradable polymers deliver a functional carrier system that can improve the constancy and accessibility of the encapsulated phytochemicals and AgNPs. This synergistic polymer–nanoparticle stage likely contributed to the antioxidant, antifungal, anti-obesity, antidiabetic, and anti-inflammatory activities detected, highlighting its potential for future biomedical applications.

CONCLUSION

Consuming an aqueous extract of *Persea americana* (avocado) leaves and a chitosan–fucoidan biodegradable biopolymer matrix, this study positively synthesized a green silver polymer nanocomposite with talented biomedical potential. GC–MS analysis and phytochemical screening complete the presence of bioactive secondary metabolites accountable for its biological properties. Antioxidant assays (DPPH, FRAP, phosphomolybdate, and H_2O_2 scavenging) demonstrated strong free radical scavenging activity. The synthesized polymer nanocomposite also exhibited important antifungal activity against *Candida albicans* and *Candida tropicalis*, potent anti-obesity activity through pancreatic lipase inhibition, and anti-diabetic activity via α-amylase inhibition. Additionally, the nanocomposite showed notable anti-inflammatory effects in red blood cell membrane maintenance and egg albumin denaturation assays, signifying its potential for future examination in PCOS-associated oxidative stress and inflammation**.** The improved biological presentation is likely qualified to the synergistic interaction between the bioactive phytochemicals, silver nanoparticles, and the chitosan–fucoidan functional polymer matrix, which acts as a biodegradable carrier and stabilizing system. Generally, the advanced biodegradable, green, functional polymer nanocomposite represents a promising platform for biomedical applications, including antioxidant, antimicrobial, anti-inflammatory, and metabolic disorder management, while offering an environmentally sustainable approach for future therapeutic and drug delivery applications.

Acknowledgment

The authors express their sincere thanks to the host institution, Dr. N.G.P. Arts and Science College, the Management, Principal, Deans, Head of the Department, Guide, and all other staff of the Department of Biochemistry for rendering all the facilities and support under the DBT-Star Scheme.

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