IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
Research Paper **© 2012 IJFANS. All Rights Reserved**, **Journal Volume 11, Iss 13, 2022**

Synthesis, Characterization OfStarch Nanoparticles From *Asparagus Racemosus* And Its Antibacterial Activity

R Rajalakshmi[1]
[1]Research scholar, Full- Time, (Reg. No 20212232262023), PG & Research Department of Botany, V.O.Chidambaram College (Affiliated to ManonmaniamSundaranar University, Tirunelveli), Thoothukudi-08.

A.Doss[2*]
[2]PG & Research Department of Botany, V.O.Chidambaram College, Thoothukudi-08.

***Corresponding Author:**A.Doss
Email: androdoss@gmail.com

Abstract

Starch nanoparticles (StNPs) have attracted growing attention due to their unique properties as a sustainable alternative to common nanomaterials since they are natural, renewable and biodegradable. The present study reports green synthesized StNPs using the old *Asparagus racemosus* plant. UV-Vis spectroscopy, FT-IR and AFM analysis revealed that synthesized StNPswith an average particle size of 6.25µm. Comparatively, the ethanol extracts showed that MIC and MBC against *Staphylococcus aureus.* Starch and aqueous extracts showed antibiofilm activity against *S.aures, lactobacillus sp.*, and *E.coli* 25µg/ml. The preliminary screening showed that the *A.racemosus* extracts of aqueous and ethanol extracts have various reducing phytochemicals, including alkaloids, flavonoids, and saponins. StNPs have been used to encapsulate bioactive compounds for controlled release in biomedical applications such as drug administration, enzyme inhibition process, and DNA precipitation.

Keywords*:Asparagus racemosus*, Starch nanoparticles, UV-Vis spectroscopy, FT-IR, AFM, Antibacterial activity (MIC & MBC), Anti-biofilm activity.

INTRODUCTION

Nanotechnology includes creating, modifying, and imaging nanostructures with diameters ranging from 1 to 100 nm (Kumar et al., 2017). Many nanotechnology components are based on chemical solubility, which can produce a product from various solvents with the necessary concentration or number of bioactive ingredients (Girija et al., 2009). Recently, starch-based nanoparticles have received increasing interest due to their ability to encapsulate, protect and control the release of bioactive ingredients. Being a biodegradable, non-toxic, abundant, and cheaper material, starch is preferred over synthetic polymers—emerging starch-based unconventional applications and technology (Neethirajan et al., 2012).

StNPs are produced through a nanotechnology technique that generates larger (Qin et al., 2016)-than single-molecule nanoparticles that are less than 1000 nm (Campelo et al., 2020). Starch is a native polymer that is abundantly available (Olayil et al., 2022) and widely used worldwide (Paulos et al., 2016). Starch nanoparticles (StNPs) are becoming increasingly popular due to their unique properties as a sustainable substitute for conventional nanomaterials because they are organic, regenerative, and biodegradable (Le Corre et al., 2010).Microorganisms might be single cells floating in the air or, more frequently, a colony of cells adhered to a substrate (Costerton et al., 1999). A *biofilm* is a complex exopolymeric substance enclosing a colony of organisms adhered to a biotic or abiotic surface (Mah & O'Toole, 2001). A biofilm consists of a community of

microorganisms adhered to a surface; within the biofilm, bacteria are cocooned in a self-produced extracellular matrix, which is composed of extracellular polymeric substances that, along with carbohydrate-binding proteins, pili, flagella, other adhesive fibres, and extracellular Deoxyribo Nucleic Acid (DNA), act as a stabilizing scaffold for the three-Dimensional (3-D) biofilm structure (Clinton & Carter, 2015). The matrix protects biofilm bacteria from exposure to innate immune defences and antibiotic treatments, enabling intimate cell-to-cell interactions and the DNA exchange that promotes the spread of drug resistance and other virulence factors. As a result, biofilm-forming pathogens persist, establishing chronic and recalcitrant infections (Fisher et al., 2010). Infections by biofilm-forming pathogens can be devastating, leading to severe symptoms and, in many instances, death (Kostakioti et al., 2013). The in vitro and in vivo efficacious of various bioactive compounds and starches from plant sources are compared (Cascioferro et al., 2020). To treat these species, starch nanoparticles are used in medicinal preparations for *Asparagus racemosus* plants. The extraction of phytochemicals is prepared using several solvents. Alkaloids, Terpenoids, Phenol, Flavonoids, Tannins, Saponins, Steroid, Phytosterols, Anthroquinones, Phlbotannins, Gums, and Oil can all be identified through these phytochemicals. Herbal medicine treats diseases or wounds with whole plants or specific plant components (Ananadharaj et al., 2021).

A genus of plants known as Asparagus belongs to the Asparagaceae family (previously included in the Liliaceae). It is a perennial herb that climbs. The genus is considered medicinal because of the presence of steroids and saponins in various parts of the plant. It uses folk to treat dyspepsia, gastric ulcers, and island as a galactagogue. Medicinal qualities generally attributed to this plant include emollient, cooling nerve tonic, constipating, and galactagogue(Sumeet & Shefali, 2008), aphrodisiac, diuretic, rejuvenating (Patel & Patel, 2013), carminative, immunostimulant, gastroprotective and antiseptic effects (Ravishankar et al., 2012).

MATERIALS AND METHODS

Collection of plant sample

The plants were collected from Thoothukudi District, on the southern coast of India. It is authenticated by Soosai Raj, Assistant Professor, the Rabiant Herbarium, St. Joseph's College, Trichy, Tamil Nadu, India; the collected leaves were thoroughly cleaned with distilled water to remove any remaining pollutants. After washing, the old plant *A. racemosus* leaves were dried in the shade for a week and ground into a powder using an electric mixer grinder.

Extract preparation

Two grams of powdered *Asparagus racemosus* plant were mixed individually with 25 millilitres of ethanol and water at room temperature (28°C). After being stirred for 48 hours, the material was filtered using Whatman No. 1 filter paper. The filtrate was collected and evaporated at room temperature (28°C). The semisolid sections are utilized in additional research.

Qualitative Phytochemical screening

Both the plant extracts were subjected to phytochemical screening using the following tests (Trease & Evans, 1983). The presence of Alkaloids, Terpenoids, Steroids, Phenol, flavonoids, tannin, Gums and mucilage, saponins, Phytosterols, anthraquinone, phlorotannins, and fixed oils were determined using the qualitative phytochemical analysis.

Synthesis of Starch Nanoparticles

The StNPs were created using, with some modifications, the technique outlined previously by Varadharaj et al. (2019). In short, 100 mL of distilled water was mixed with 5 g of dried potato starch. The mixture was continuously stirred mechanically for 150 minutes at room temperature until a homogenous solution formed. 20 mL of plant extract was added to this mixture dropwise while being vigorously stirred by a machine continuously. For four hours, the mixture was agitated to make it clear. After that, it was centrifuged for 20 minutes at 10,000 rpm. After removing the

supernatant, 70% ethanol and distilled water were used to purify the settling nanoparticles, eliminating water and unreacted substances. Using a lyophilizer, the StNP solution was freeze-dried. The lyophilized StNPs were stored in a container for further analysis and testing.

Characterization of nanoparticles

Synthesized starch nanoparticles were analyzed using a NanoDrop 2000C Spectrophotometer (Thermo et al., USA), with the absorption spectrum measured between 200 and 800 nm, using double distilled water as a reference. The Varian FTIR 640 spectrophotometer was used for FTIR analysis in the 400-4000 cm−1 range. Fourier-transformed infrared radiation spectroscopy studies were performed to determine the probable biomolecules responsible for reducing Starch ions and the capping of reduced StNPs synthesized by the plant extract. A small sample volume was spread on a glass coverslip surface mounted on the AFMs and dried with nitrogen flow at room temperature. The blank, in this particular instance, was de-ionized water. Atomic force microscopy (Model-Nanosurf easy scan 2 AFM, manufactured in Switzerland) was used to characterize the samples from the maximal time point of starch nanoparticles generation. The samples were dried by air and examined for size, morphology, and starch agglomeration. AFM images were captured using silicon cantilevers in contact mode with a force constant of 0.02–0.77 N/m and a 10–15 nm tip height. Images from an AFM represent data in three dimensions, which makes it possible to estimate the height of the nanoparticles quantitatively (Mucalo M et al., 2002; Vasenka J et al., 1993).

Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC value of the extract was determined as the lowest concentration that completely inhibited bacterial growth after 48 hr of incubation at 37^{o}C. For the determination of MBC, a portion of liquid (5 µl) from each plates well that exhibited no growth were taken and then incubating 37^{o}C for 24 hr. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC. Positive and negative cultures were also prepared.

Anti-biofilm activity

The study evaluated the impact of crude extracts and nanoparticles on biofilm formation in fresh nutrient broth. Tubes were cultured in varying concentrations, and the biofilm formed was stained with crystal violet. The dye was solubilized with ethanol, and absorbance was measured using spectroscopy. Biofilm determination was determined using the formula SBF = (AB - CW)/G, where SBF represents specific biofilm formation, AB represents attached bacteria, CW represents control wells, and G represents cell growth in broth.

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical study found that *A.racemosus* leaf extracts contain alkaloids and flavonoids, with ethanol containing terpenoids, flavonoids, alkaloids, steroids, and phytosterols, while aqueous extracts contain alkaloids, flavonoids, and saponins. The presence of the tannins, alkaloids, and saponins in the extract shows that each of these phytochemicals is a significant bioactive component of the plant's roots and may contribute to the part of the plant that acts as a medicine. According to Shevale et al. (2015), a phytochemical examination of *Asparagus racemosus* root extract revealed the presence of significant bioactive secondary metabolites, including tannins, flavonoids, steroids, saponins, and amino acids. In this study, ethanol extract contained steroids, amino acids, carbohydrates, tannins, saponins, and flavonoids. According to Sivakumar and Gajalakshmi (2014), the *A. racemosus* root extract's qualitative phytochemical screening of the presence of alkaloids, steroids, flavonoids, glycosides, saponins, and terpenoids was demonstrated by the methanol extract of the root of *A. racemosus*.

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Table1: Preliminary phytochemical screening of crude extracts of *A.racemosus*

Phytochemical	Ethanolextract	Aqueousextract
Alkaloids	+	+
Terpenoids	+	_
Phenol	_	_
Flavonoids	+	+
Tannins	_	_
Saponins	_	+
Steroids	+	_
Phytosterols	+	_
Anthroquinones	_	_
Phlobatannins	_	_
Gums and mucilages	_	_

Synthesis and characterization of starch nanoparticles

Starch nanoparticles were synthesized by reducing starch solution with an aqueous extract of *A.racemosus*. Several techniques have been reported worldwide to characterize herbal and medicinal plant-based nanoparticles.The formation of starch nanoparticles was verified spectroscopically by analyzing the surface plasma resonance (SPR) on the nanoparticles with UV-visible spectroscopy, which made it possible to verify their synthesis. The UV peaks for the starch NPs synthesized by *A.racemosa*are shown in **Figure 1**. The starch nanoparticles created using *A.racemosa* extract showed an absorbance maximum 280 nm, that peak depends upon the nanoparticles colours. The conducting electrons in the biosynthesized nanoparticles display oscillation due to surface Plasmon resonance, bringing about an absorption peak at a specific wavelength. The FTIR spectra of starch nanoparticles are illustrated in **Figure 2**. The results showed that the chemical structure of starch did not change after hydrolysis. All spectra exhibited the vital broadband of OH stretching vibration of the hydroxyl group in the glucose units at 3165cm1. According to Varadharaj (2019), the O-H stretching of amylopectin is responsible for the broadband seen at 3348 cm-1, and the creation of intra- and intermolecular hydrogen bonds has been suggested as the cause of the band's width. The bending vibration of C-OH & C-O-C, which revealed the glycosidic solid linkages, is responsible for the peak at 1025.27 cm-1. Yan et al. (2022) studied the stretching vibration of the C–O–C is represented by 1154 cm−1, while the C–O linked is represented by 1080 cm−1 (Li et al., 2019). The peaks corresponding to the stretching of the anhydroglucose ring are 869.92 cm-1, 763.68 cm-1, and 661.52 cm-1. According to Chao Qiu et al. (2016), it was noticed that the peaks at 1637.74 cm-1 stretched into bands made up of protein aid and ester. The firmly bound water found in the nanoparticles was responsible for the peak at 1640 cm-1 (Table 2). The starch nanoparticles were characterized by Atomic Force Microscopy (AFM) for the detailed size and morphology of starch. The topographical images of starch nanoparticles synthesized by *A.racemosa* extract are shown in **Figure 3**. The particle size of the starch nanoparticles was found to be 6.25µm, corresponding to *A.racemosa*.Szymońska et al. (2009) reported that the granular potato starch size was from 210 to 258 nm and had a length between 1.56 µm. According to Neethirajan et al. (2012), J. Buckwheat of AFM image shows the granules are a compact arrangement of starch along with few protein bodies and growth rings, visible in the error-signal mode images and topography image of approximately 14nm.

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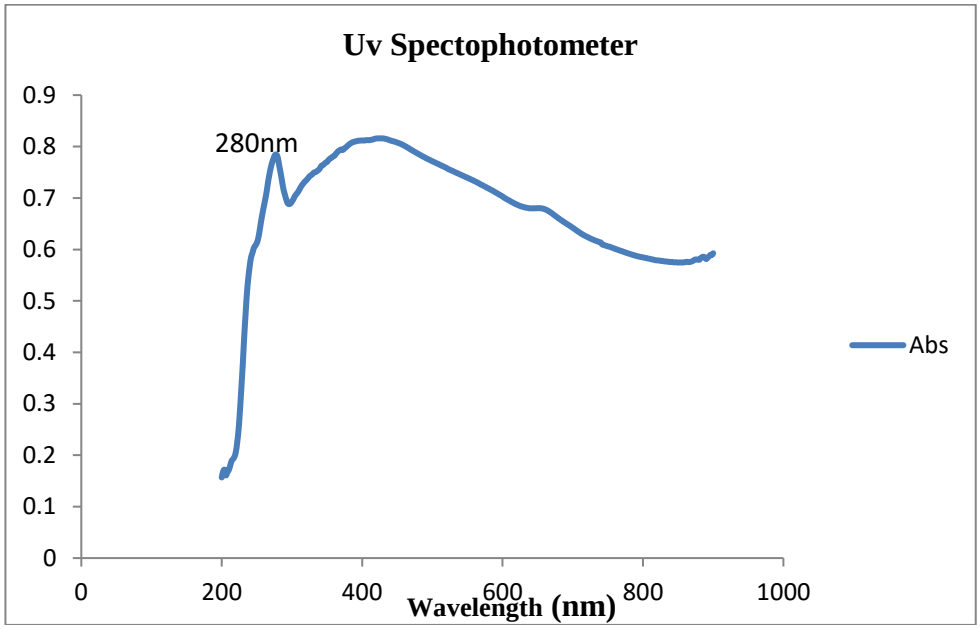

Figure 1: UV-Visible Spectrophotometer

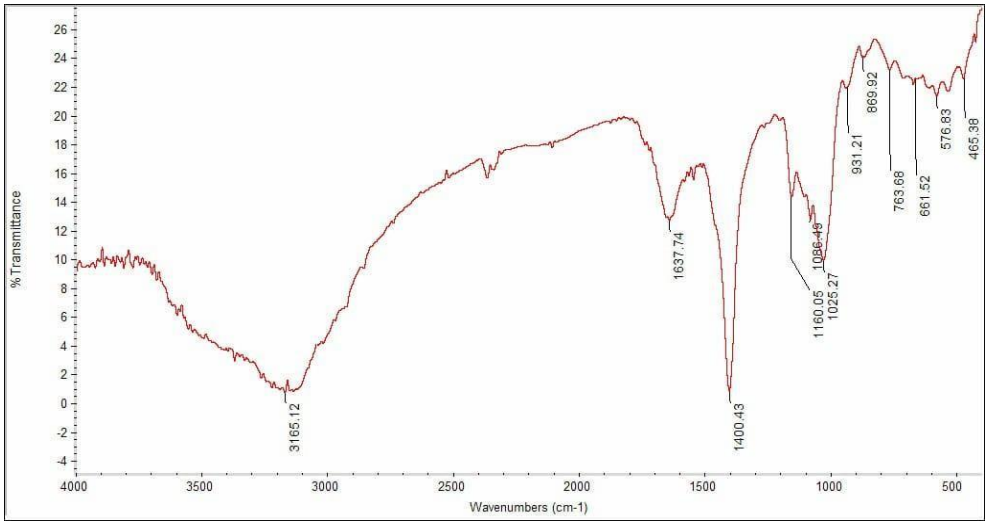

Figure 2: FT-IR Spectrum

Table 2: Functional Group of Starch Nanoparticles in FTIR Analysis

StNPs Peak	**Functional Group**
465- 578	C–X stretch
661	C–H
869- 873	=C–H
921-931	=C–H
1025-1063	C-O-C on starch chain
1156-1160	C–O ether bond
1420-1421	angular deformation of C–H
1637-1639	C=C stretch (conjugated)
3165-3338	carboxylic acid with O–H stretching vibrations

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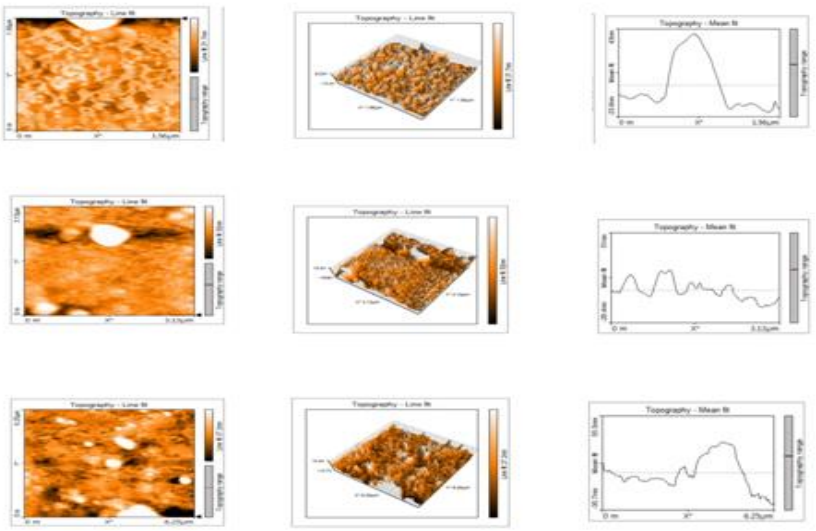

Figure 3: AFM Structure of Starch Nanoparticle

Antibacterial activity

The results of the antibacterial activity of the plant extracts—ethanolic and StNPs—are displayed in (Tables 3, 4 and 5). With MICs and MBC of 8 mg/µl, the ancient *A.racemosus* old plant extracts demonstrated antibacterial efficacy against the fast-growing bacteria. There is a notable appearance of ethanol extracts in the MICs and MBC. According to Thakur et al. (2015), the methanolic extract of *Asparagus racemosus* root had an active antibacterial effect against these bacteria at concentrations of 12.5 mg/ml and 25 mg/ml, respectively, based on its MIC and MBC. It can also be tested for this activity against other microorganisms that cause tooth infections. The low MIC and MBC against *Actinomyces howellii* demonstrate the effectiveness of the plant extract against gram-positive bacteria.

Table3: Minimum inhibitory concentration of Ethanol Extract of *A.racemosus*

Name of the Culture	Concentration of ethanol extract (µg/mL)	MIC	MBC
Staphylococcusaureus	8	+	16
	4	_	ND
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND
Lactobacillussp.,	8	+	16
	4	+	8
	2	+	4
	1	+	2
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND
Escherichiacoli	8	+	16
	4	+	8
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND

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	0.125	_	ND

Table4: Minimum inhibitory concentration of Aqueous Extract of *A.racemosus*

Name of the Culture	Concentration of aqueous extract (µg/mL)	MIC	MBC
Staphylococcusaureus	8	_	ND
	4	_	ND
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND
Lactobacillus sp.,	8	_	ND
	4	_	ND
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND
Escherichia coli	8	_	ND
	4	_	ND
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND

Table 5: Minimum Inhibitory Concentration of Starch Nanoparticles of *A.racemosus*

Name of the Culture	Concentration of Nanoparticles (µg/mL)	MIC	MBC
Staphylococcus aureus	8	_	ND
	4	_	ND
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND
Lactobacillus sp.,	8	_	ND
	4	_	ND
	2	_	ND
	0.25	_	ND
	0.125	_	ND
Escherichia coli	8	_	ND
	2	_	ND
	1	_	ND
	0.5	_	ND
	0.25	_	ND
	0.125	_	ND

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Antibiofilm activity of crude extracts and starch nanoparticle

The starch nanoparticles made from the ancient plant extract *A.racemosa* exhibited antibiofilm activity at 25 µg/ml doses against *S. aureus and E. coli*. According to their MIC and MBC, the biofilm inhibitory assay, aqueous extract, and starch nanoparticles were assessed at various concentrations. At all tested concentrated levels, there was more than 50% suppression of biofilm (Table 6 & 7). The starch nanoparticles inhibits *staphylococcus aureus and E.coli*, at 90% and 96%, while the highest biofilm inhibition of aqueous extract at Lactobacillus sp., is 99%. Nartey et al. (2021) *Averrhoa carambola* L. fruit and leaf essential oils were evaluated at the concentration of MIC /32, and a 52% biofilm inhibition was observed. The maximum biofilm inhibition of 77.94% was observed at the MIC of the fruit essential oil.

Table6: Anti biofilm activity of Starch Nanoparticles of *A.racemosus*

S.NO	**Name of the bacteria**	***control***	**Concentration of Starch nanoparticles (µg/mL)**				
			***5* (µg/mL)**	***10* (µg/mL)**	***15* (µg/mL)**	***20* (µg/mL)**	***25* (µg/mL)**
1	*S.aureus*	*0.56*	*23*	*52*	*64*	*77*	*90*
2	*Lactobacillus sp.,*	*0.67*	*64*	*48*	*56*	*52*	*23*
3	*E.coli*	*0.75*	*96*	*54*	*36*	*27*	*82*

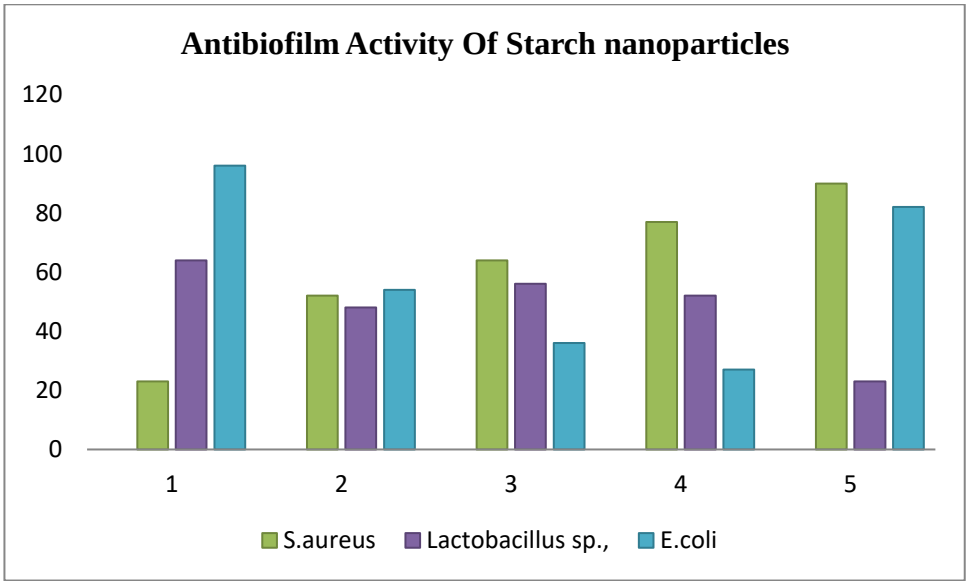

Figure 4: Antibiofilm activity of Starch Nanoparticles of *A.racemosus*

Table 7: Anti biofilm activity of aqueous extract of *A. racemosus*

S.NO	**Name of the bacteria**	***control***	**Concentrationof aqueous extract (µg/mL)**				
			***5* (µg/mL)**	***10* (µg/mL)**	***15* (µg/mL)**	***20* (µg/mL)**	***25* (µg/mL)**
1	*S.aureus*	*0.76*	*73*	*49*	*54*	*29*	*39*
2	*Lactobacillus sp.,*	*0.45*	*99*	*50*	*83*	*47*	*37*
3	*E.coli*	*0.65*	*40*	*57*	*61*	*21*	*57*

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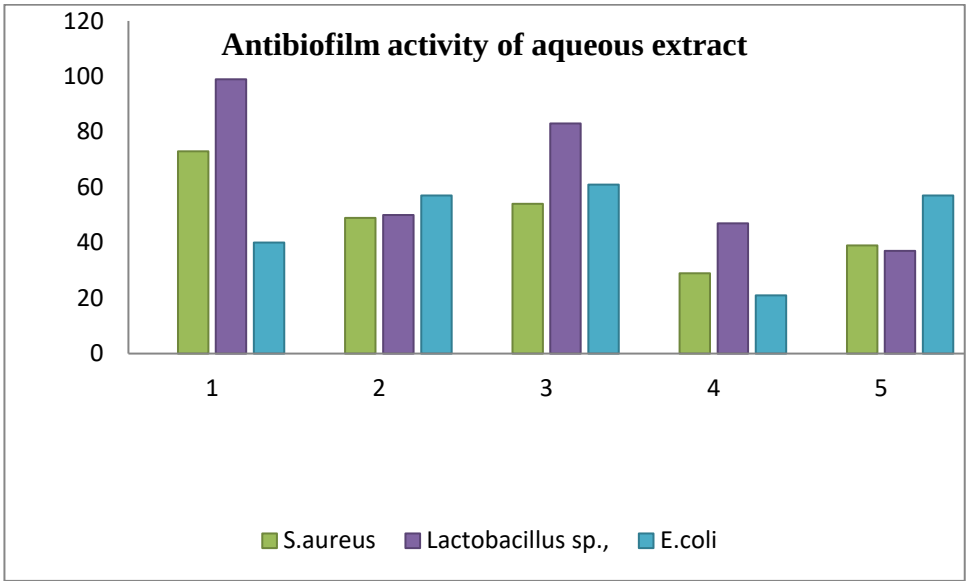

Figure5: Antibiofilm activity of aqueous extract of *A. racemosus*

CONCLUSION

An old plant extract from *Asparagus racemosus* can produce StNPs in a stable solution. By supporting the synthesis of nanoparticles by UV spectrum absorption at wavelengths between 200 - 300 nm and by causing colour changes in reactions, StNPs improve the efficiency of synthetic processes employing naturally occurring, environmentally friendly materials as an alternative to chemical synthesis. Several functional groups, such as alcohols, ketones, alkenes, and carboxylic acid, present in *A.racemosus* plants as secondary metabolites, synthesize nanoparticles. Additionally, it was established that the composite release of starch at a core has antibacterial activity and is effective against both Gram-positive and Gram-negative pathogenic microorganisms. The current approach is a simple, cost-effective, secure, and non-toxic chemical one.

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