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Original Article

Pharmacology Section

Antibacterial and Antibiofilm Activity of Silver Nanoparticles Synthesised by Beetroot Containing Betalains Pigment on Clinical Bacterial Isolates

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ABSTRACT

Introduction: Artificial colours have been used in foods for many years with adverse side-effects. As a result, many studies have focused on natural dyes, and interest in natural dyes is increasing every day due to the lack of side-effects. The betalains are pigments, which are present in the *Beta vulgaris* L (red beet) roots; these are exploited for native colouring and additive agents in food. These compounds possess many desirable properties such as antioxidant and antimicrobial activity etc., Nanotechnology is currently being used to enhance plant medicinal applications. It is an environmentally non toxic and low cost method.

Aim: To examine the role of beetroot containing betalain on synthesising Silver Nanoparticles (AgNPs) and to determine the antibacterial activity.

Materials and Methods: This in-vitro study was carried out in Department of Botany, KK College in Namakkal, Tamil Nadu during the period of December 2018 to December 2020. The 2mM of AgNPs was utilised for the preparation of nanoparticles with beetroot containing betalains. The characterisation of synthesised AgNPs was done by Fourier Transform Infrared spectroscopy (FTIR), Ultraviolet-Visible (UV-Vis) spectroscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). In addition, the antibacterial activity of AgNPs were evaluated by the agar well-diffusion method.

Results: In this study, the highest concentration of betalains observed at pH 5 in both solvents [ethanol (154.4 mg/100 mg) and water (131.2±0.15/100 mg)] was recorded. The acetone recorded a maximum of 143.8 mg/100 mg at pH 4. Bio sourced AgNPs had antibacterial activity against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeruginosa, Proteus sp, Salmonella sp and Acinetobacter baumannii*. Furthermore, synthesised AgNPs inhibited the biofilm formation in all isolates.

Conclusion: This study reveals that AgNPs exhibit strong antimicrobial activity so that they can be developed as new types of antimicrobial agents to treat bacterial infections, including biofilm bacterial infections, and are attractive and environmentally friendly.

Keywords: Additives, Antibacterial, Microscopy, Nanotechnology, Natural pigment

INTRODUCTION

The incidence of microbial infections has increased dramatically in recent decades. Continued use of antimicrobial drugs in infection treatment leads to resistance among different microbial strains [1]. As a result of unrestrained exploitation and misuse of antibiotics, bacteria exhibit antimicrobial resistance [2]. Bacteria that are resistant to multiple drugs cause infections which cause bountiful effects, which includes the raise in mortality and morbidity rates, an extension of the hospitalisation period and economic loss [3]. In this situation, there is an urgent need to develop a new and natural antibiotic as there is a growing concern about pathogens in the food that are resistant to many drugs.

In recent decades, there has been a growing interest in the development of new and effective antibiotics against infections caused by antibiotic-resistant bacteria. In that sense, it is necessary to do a lot of research on nanoparticle technology to inhibit the bacteria that cause the bad effects. In recent years, AgNPs have received great attention from many researchers engaged in multidisciplinary research due to their unique characteristics and wide range of applications [4], like food science, medical, agriculture, and agricultural technologies. The use of nanoparticle technology in the medical world is now increased, especially to suppress the monster of microbes.

Number of methods utilised for the synthesis of nanopartilces, however, physiochemical photochemical reduction and so no electrochemical methods were failure because of cost of chemicals, need high energy, time consuming, and difficulty in waste purification of hazardous chemicals [5]. Therefore, there is a growing need to use safe and green package routes for the economy and environment in the synthesis of nanoparticles. On the other hand, the green set of AgNPs that use a variety of microorganisms, plants and algae is a natural, bio-friendly and eco-friendly process [6].

Some plant pigments have produced expectations of health benefits, such as anthocyanin pigment products (Ex. corn), which are expected due to their purple colour and antioxidant health benefits [7]. Many studies have been conducted on the antioxidant and antibacterial properties of natural pigments used as food colourants [8-10]. Beetroot contains significant amounts of pigments such as betaxanthins and betacyanin, which belong to the betalain family, a group of water-soluble nitrogen-containing pigments derived from betalamic acid, which has long been used in traditional medicine to treat many ailments [11].

Studies show that beetroot contains betalains, which acts as a protective molecule, and beetroot is said to have anti depressant, hepatoprotective, antihypertensive, antioxidant, antihyperlipidemic, antiradiation and many therapeutic benefits and immunostimulatory effects [12-14]. Furthermore, they have anticancer, immunomodulatory, anti-inflammatory, antimutagenic, antimicrobial and antifungal properties, and they can also be used as expectorants and carminatives [15]. Number of previous studies demonstrated that

betalains are active against microbes [16,17], however no one has done much research on the nanoparticles using beetroot containing betalains as opposed to antimicrobial activity. Therefore, it is very interesting to explore whether the integrated AgNPs' inhibitory effect on bacteria will be more powerful in the field of nanotechnology and play their role in an environment friendly and secure manner. The present study aimed to synthesise AgNPs using betalains and evaluate their antibacterial efficacy against bacterial pathogens.

MATERIALS AND METHODS

Collection of Beetroot and Preparation

This in-vitro study of beneficial activity of red beet was carried in Department of Botany, KK College in Namakkal, Tamil Nadu, India, from December 2018 to December 2020. The fresh red beets were acquired from local vegetable shops and thoroughly washed to remove any soil. With a sterile knife, vegetables were cut into pieces and weighed them. Red beets (200 gm) were mashed in 1 liter of ethanol (acidified with 2% citric acid) using a blender and left at room temperature for 15 minutes. A rotary vacuum evaporator at 40°C was utilised to filter and concentrate the extract under vacuum, which was then used in further research [16].

Estimation of Betalain

The amount of betalain in each solvent vis ethanol, acetone and water were estimated as mg/100 g basis. The diluted content was measured at 535 nm wavelength and it is calculated using the given formula as mg betalains/100 g as given by Castellar R et al., [17].

Bt (or Bx) content (mg/L)=A×DF×MW×1000/ε×i

for Bt A536 nm and ε=60,000 (molar extinction coefficient in Lmol–1 cm–1); for Bx A485 nm and ε=48,000 (Lmol–1 cm–1); DF=Dilution Factor; Molecular Weight (MW)= 550 g/mol (for Bt) or 339 g/mol (for Bx); i=path length (1 cm).

Betalains Estimation at Different pH and Solvents

The optimised pH value was determined using the buffer 0.1 M HCl, which was added gradually until the desired pH (2, 3, 4, 5 and 6) is reached and left undisturbed at room temperature for 30 min. The betalain content was calculated as per estimation procedure [18].

Betalains Estimation at Different Temperature and Solvents

The optimised temperature was determined for extraction of betalains. Samples of 5 mL were held for 30 minutes in thermostatically controlled water bath at 25, 35, 45 and 55°C, and cooled immediately in an ice bath. The betalain content was calculated as per estimation procedure [18].

Synthesis of Nanoparticles

Betalains (100 mg/1 mL) was mixed to 10 mL aqueous solution of $AgNO_3$ (2 mM) and stirred continuously for 10 min at room temperature and it was turned to brown in colour after 7 hour which give silver colloid. The synthesised nanoparticles were stored in the refrigerator for the antibacterial activity test and further analysed by using UV-Vis spectrophotometer SEM and TEM.

Characterisation of Nanoparticles

UV/VIS/Spectrometer and FTIR

The UV spectrophotometer and FTIR analysis was carried in Biodeavour, Chennai, Tamil Nadu. The absorption spectra of aqueous precursors and synthesised AgNPs were recorded with UV/VIS/ spectrometer. The FTIR spectrum of aqueous solution of AgNPs were recorded using Thermo Scientific Nicolet iS50-India with resolution at 4.000 from 400 to 4000 nm.

SEM with Energy-Dispersive X-ray spectroscopy (EDX)

The UV SEM and TEM analysis was carried in Biodeavour, Chennai, and Tamil Nadu. The morphology of the green synthesised AgNPs with betalanin was analysed using SEM coupled with EDX. The AgNPs solution was centrifuged at 10,000 rpm for 20 min and drop coated on to thin glass film fabricating and allowing water to completely evaporate and analysed using Zeiss EVO 18 at a voltage of 20kV. The elemental identification and quantitative compositional information was obtained using EDX.

TEM with EDX

For microscopy, a single drop of synthesised AgNPs were carefully placed on a copper coated grid, and the samples were dried for 4 min and imaged for their size and shape on (Spherical in cluster) operating at a voltage of 200 kv. Diameter (D) was calculated for each nanoparticle sample by averaging 200 particles from the TEM images using Image J software (National Institutes of Health, USA). The elemental identification and quantitative compositional information was obtained using EDX.

Collection of Clinical Isolates

The clinical isolates of *E. coli, E. faecalis, P. aeruginosa, A. baumannii, K. pneumoniae, Salmonella sp, Proteus sp,* and *S. aureus* were procured from Microtech clinical laboratory, Coimbatore. Based on the commonly available bacterial isolates were obtained and used in this study. The collected isolates were confirmed with selective media and stored in nutrient agar slant kept in refrigerator condition for further studies.

Determination of Antibacterial Activity of Betalains

A fresh inoculum from an overnight bacterial culture (108 CFU/mL) was swabbed on Mueller Hinton agar plates to cover the complete surface of the medium. In agar plates, wells of approximately 6 mm diameter was made with a sterile stainless steel borer. A 100 µL of different concentrations of betalains was filled in well with the help of micropipette and one well filled with ethanol and another well filled with ampicillin (10 mcg). The petriplates were kept for incubation at 37°C for 24 hours. After incubation, the microbial growth was determined by measurement of the zone of inhibition around each well and recorded in mm [19]. Same procedure was utilised for the determination of antibacterial activity of AgNPs and compared the zone of inhibition between the AgNPs and non AgNPs.

Antibiofilm Activity Determination with AgNPs

According to Gurunathan S et al., and Barapatre KR and Jha AH, procedure [20,21], antibiofilm activity was determined with AgNPs and non AgNPs of betalains. Along with 180 µL of Mueller Hinton Broth, add 10 µL of the test pathogens (OD=1.0, 600 nm) in each well of titer plate. Following that, 10 µL containing of AgNPs was added to each well and mixed well gently with the rotator of the plant. Then plate was incubated at 37°C for 24 hours. The mixture without bacteria was considered as control. The contents in the well were discarded upon incubation and wash gently with Phosphate Buffered Saline (PBS, pH 7.2) for the removal of bacterial cells which were free floating and non adherent to the bottom of wells. The wells were then air dried for 45 minutes at room temperature. The remaining bacterial cells that are adherent were fixed with 2% w/v sodium acetate and fixed in wells. The well of plate was incubated in dark for 30 min for staining with crystal violet stain (0.1%, w/v). The deionised water was used to rinse the wells for the removal of excess dye. The plates were air dried and add 200 µL of ethanol (95%, v/v) in all the wells and measure the absorbance at 620 nm. The following equation was used to calculate the percentage of inhibition of biofilm formation:

Percentage of % biofilm inhibition=[1–(OD620 of cells treated with AgNPs or plant extracts/OD620 of the non treated control)×100].

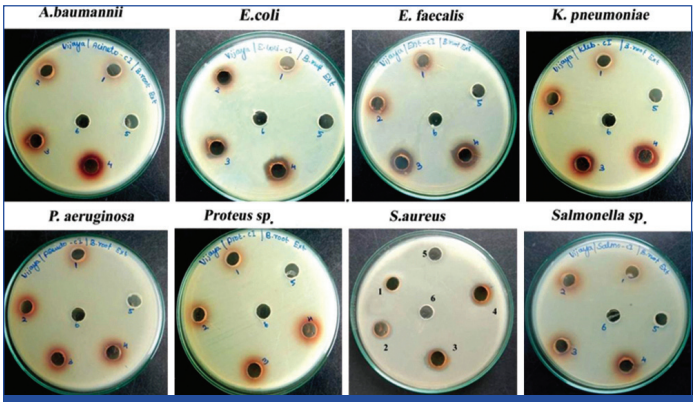
STATISTICAL ANALYSIS

The data were entered into Microsoft excel 2007 version and further analysed using Statistical Package for Social Sciences software (SPSS) version 20.0, (Chicago IL, USA). For descriptive analysis, the categorical variable was analysed by using percentage and the continuous variable was analysed by calculating mean±standard deviation. For inferential analysis, the numerical data were analysed using t-test and the categorical data were analysed using Chi-square test and a p-value <0.05 was considered as statistically significant.

RESULTS

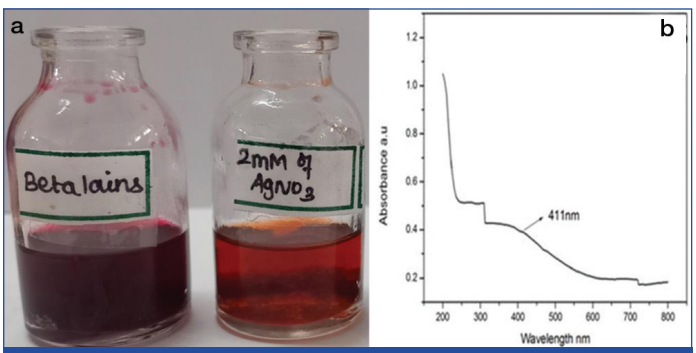
In this study, an attempt was made to identify and characterise the betalains pigment present in *Beta vulgaris* L and its effective use in the therapeutic field. In this study, the highest concentration of betalains observed at pH 5 in all two solvents (ethanol (154.4 mg/100 mg) and water (131.2±0.15/100 mg) was recorded. The acetone recorded a maximum of 143.8 mg/100 mg at pH 4. The optimum temperature in this investigation was 35°C, with a maximum betalain concentration of 127.5 mg/100 mg when ethanol was used.

Presently, all isolates were suppressed with betalain however *Proteus sp* was not inhibited. The zone of inhibition was ranged from 8.2±0.52 mm to 14.5±0.52 mm. Antimicrobial effect appears to be dose dependent, that is as concentration rises, bacterial inhibition rises as well. Among the isolates, *E. faecalis* was highly suppressed even with 1mg of betalain and lowest inhibitory activity was observed against *P. aeruginosa*, which exhibiting zone of inhibition was 8.2±0.52 while using 8 mg of betalain. The negative control of ethanol and positive control of ampicillin showed no zone of inhibition against bacteria [Table/Fig-1].



[Table/Fig-1]: Antibacterial activity of betalains against clinical isolates, 1-1 mg, 2-2 mg, 3-4 mg, 4-8 mg, 5-Ampicillin, 6-Ethanol (Control).

The colour change of the colourless $AgNO_3$ solution to a brown suspension of silver nanoparticles, as shown in [Table/Fig-2a], is one approach for confirming the creation of nanoparticles. UV-visible spectrum of the biosynthesis of silver nanoparticles using the betalain showed a peak at 411 nm corresponding to the plasmon absorbance of silver nanoparticles for the tested sample [Table/Fig-2b]. Metal

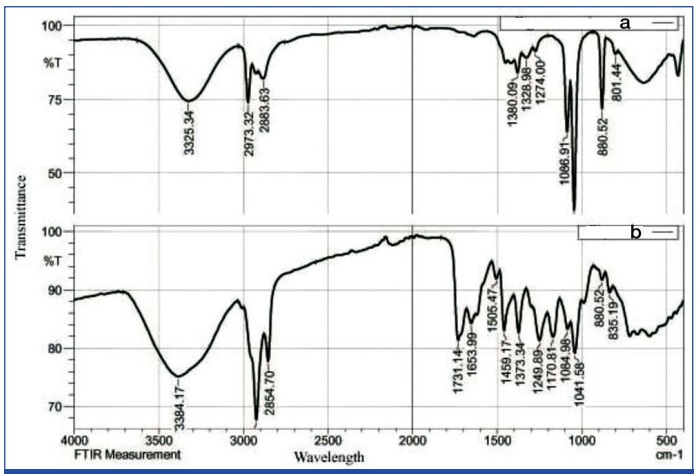


[Table/Fig-2]: Change in the colour of the solution from brown to dark brown. Betalanin solution; a) change in the colour of the solution indicate as AgNPs synthesis; b) UV-Vis spectra of synthesised AgNPs of Betalain.

nanoparticles contain free electrons, which provide a Surface Plasmon Vibration (SPR) absorption band, which causes mutual vibration of the electrons of metal nanoparticles echoing with the light wave. The appearance of the peaks shows the surface plasmon vibrations of silver nanoparticles.

FTIR Analysis

The FTIR spectra of betalain aqueous extract was shown in [Table/Fig-3a,b]. The [Table/Fig-3a] demonstrates the broad absorption frequency at 3325.34 cm^{-1} which might due to the presence of -OH functional group. The sharp absorption peak at 2973.32 cm^{-1} corresponds to the -C-H stretching frequency of alkanes. The C=O stretching frequency of betalain was found at 1380.09 cm^{-1}. The bands at 1086.91 and 1045.44 cm^{-1} might represents the symmetric and asymmetric C-O-C stretching frequency and primary alcohol stretching frequency. The absorption peak at 880.52 cm^{-1} relates the C-COOH stretching frequency.

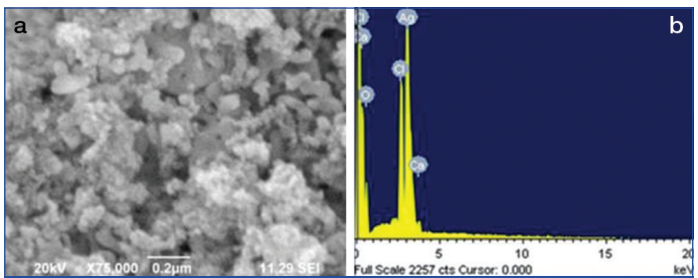


[Table/Fig-3]: FTIR spectra of; a) Betalain extract; b) Betalain with NPs.

The FTIR of synthesised nanoparticles was shown in [Table/Fig-3b]. Comparison of [Table/Fig-3a,b] represented a slight shift in the intensity and positions of the absorption peaks which is due to the formation of nanoparticles. The displacement of peaks at 3384.17, 2854.70 cm^{-1} and the less intense peaks 1084.98 cm^{-1} and 1045.44 cm^{-1} might be due to the breakdown of hydrogen bond that plays a vital role in the reduction of nanoparticles. The disappearance of peak at 880.52 cm^{-1} might demonstrates the carboxylated nanoparticles.

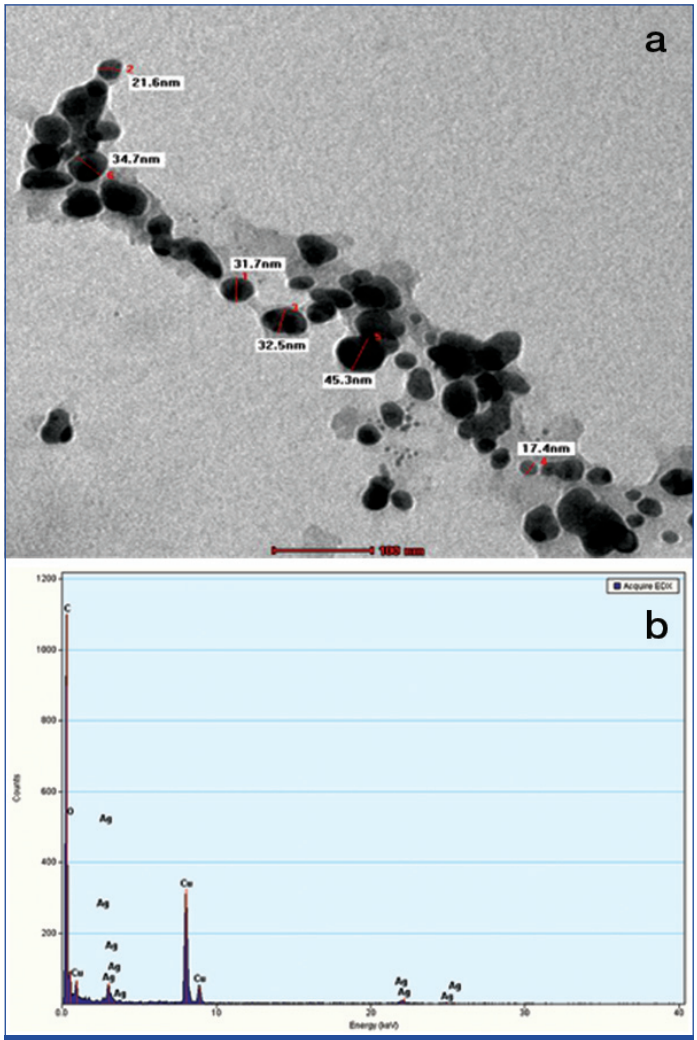
The scanning electron micrograph revealed the morphology of the biosynthesised silver nanoparticles to be relatively spherical. [Table/Fig-4a] represents the green synthesised AgNPs of betalain. [Table/Fig-4b] illustrates standard EDX spectrum recorded on the examined sample. In the middle part of the presented spectrum, a peak of Ag is located between 2 kV and 4 kV and maxima are directly related to the Ag characteristic line. Quantitative analysis proved silver contents of 67.93% in the examined samples. The EDX spectrum also reveals other peaks viz., oxygen (14.86%), followed by chloride (15.43%) and calcium (1.79%).

TEM micrographs provided additional insight into the morphology and particle size distribution profile of the green synthesised silver



[Table/Fig-4]: a) SEM images of silver nanoparticles synthesised by betalain; b) EDX spectrum of synthesised AgNPs using by betalains.

nanoparticles. The analysis of data obtained from TEM micrographs of silver nanoparticles revealed that the particles are spherical and the size ranged from 17.4-45.3 nm with an average particle size of 30.53 nm [Table/Fig-5a]. TEM/energy-dispersive X-ray spectroscopy (EDX) imaging confirms the accumulation and dissolution of AgNPs and the spectrum [Table/Fig-5b] demonstrates the presence of silver.

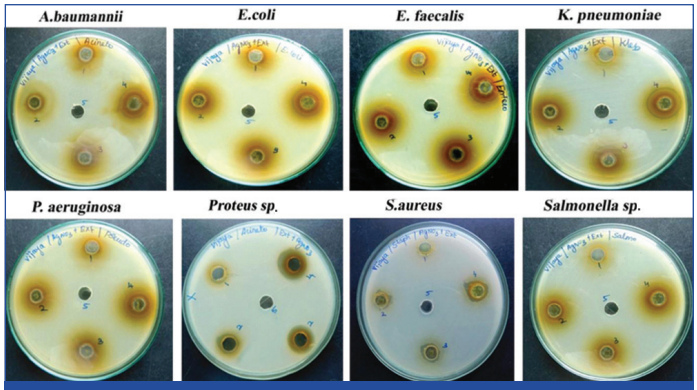


[Table/Fig-5]: a) TEM pictures of spherical silver nanoparticles; b) EDX graph of silver nanoparticles of betalain. TEM/energy-dispersive X-ray spectroscopy (EDX) imaging confirms the accumulation and dissolution of AgNPs and the spectrum demonstrates the presence of silver.

The synthesised silver nanoparticle was subjected to antimicrobial activity against above mentioned isolates. The inhibitory activity was improved while using the AgNPs than crude extract. The zone of inhibition ranged from 8.4±0.12 mm to 21±1.42 mm. AgNP betalain suppressed all bacterial isolates at 2 mg concentration. Among the 8 genera, *A. baumannii* was highly suppressed, which exhibited the zone of inhibition range from 15±1.42 mm to 21±1.42 mm. The lowest inhibitory activity was observed in *Proteus* sp. and *S. aureus*. While control $AgNO_3$, inhibited none of the isolates tested [Table/Fig-6]. This study finding revealed that AgNPs betalain extract demonstrated more inhibitory activity than the crude extract against all the gram positive and gram negative isolates.

In the present study, Minimum Inhibitory Concentration (MIC) of AgNPs were observed through titer plate well method. The 0.25 mg of concentration was recorded as the MIC for *E. faecalis, A. baumannii* and *K. pneumoniae,* 0.5 mg of for *E. coli, Proteus* sp and *S. aureus* and MIC of 1 mg concentration was recorded for *P.aeruginosa* and *Salmonella* sp.

An important finding of this study is that AgNPs inhibit the formation of biofilms of bacterial isolates, due to the inhibitory effect of AgNPs on the flagella. In the current research, the in-vitro antibiofilm activity of AgNPs was determined against all bacterial isolates. Treatment



[Table/Fig-6]: Antibacterial activity of AgNPs synthesised with betalain and active against clinical isolates, 1-1 mg, 2-2 mg, 3-4 mg, 4-8 mg, 5-$AgNO_3$ (Control).

of *S. aureus* for 24 hours with AgNPs (2 mg/mL) synthesised using betalanin extract, reduced biofilm formation by 78.2%, same time while using non AgNPs, 36.4% of biofilm formation was reduced.

The mean biofilm formation of AgNPs (54.1%) was greater than biofilm formation of plant (26.4%). The mean difference between the two groups was 27.7% (p-value=0.0024). Thus, this study conclude that the difference in mean biofilm formation was statistically significant [Table/Fig-7].

	Biofilm formation with AgNPs		Only plant extract		
Variable	Mean	Std. Deviation	Mean	Std. Deviation	p-value
Isolates	54.1%	15.7	26.4%	5.9	0.0024

[Table/Fig-7]: Inhibition of biofilm formation with plant extract and AgNPs. A p-value <0.05 was considered as statistically significant

DISCUSSION

In this study, betalains were extracted best at a pH of 5 which was also correlated with Sabarudin NA et al., who reported that the optimal pH value for extracting betacyanin from *Bougainvillea bracts* was pH 4-5 and the concentration was 10.67 g/L [22]. The Kugler F et al., reported that betalain was stable in the pH range from 3.0 to 7.0, which was in support to the current study [23]. The temperature is the most important factor during the extraction and concentration processes of plant pigments. The results of the current investigation confirm with the Tang CS and Norziah MH results, in which the maximum pigment betalains was detected at 25 to 30°C and if there is increase in temperature, there is reduced betalains retention [24]. Roy K et al., reported that the extraction of betalains from red beet was optimal at 40°C [25]. Earlier study by Barrera FAG et al., reported that betalain degradation occurs with increasing in temperatures can be attributed to by isomerisation, decarboxylation or cleavage by heat or acids [25].

When compared to acetone, water and ethanol proved to be the most effective extraction solvent in this investigation. Ravichandran K et al., and Righi Pessoa da Silva H, said that the concentration of ethanol in the extraction solvent had the greatest impact on the extraction of betalain [27,28]. The findings of this study was inconsistent with those observed by Das M et al., in which they reported that the concentration of betalain in water extract was higher than in ethanol extract [29].

The positive effects of numerous plants containing betalains have been proven in a number of studies [30-33]. However, there has been little research on betalain's antibacterial action in beetroot. Canadanović-Brunet JM et al., claimed that beets containing betalain have antimicrobial properties [34]. According to Jacob SJP and Shenbagaraman S, betalains found in red beets have an important role as an antioxidant, antiviral, antibacterial, hepatoprotective, and anticancerous agent [35]. In the present study, 8 bacterial species of 18 isolates were procured and confirmed with chromogenic media. Those confirmed isolates were subjected for the determination of antibacterial activity of betalain.

Canadanović-Brunet JM et al., have demonstrated the antibacterial activity of beetroot pomace extract against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa* supports the present study findings [34]. Findings by Saani M and Lawrence R, on the beetroot extracts showed potential activities against the gram positive and gram negative bacteria such as, *B. subtilis, S. aureus, E. coli and S. dysenteriae* also was in concordance with the present study [36].

A change in colour of the mixture indicates the reduction of silver ions (Ag^+) to atomic silver (Ag0) which coalesce to form SNPs [37]. Likewise the colour change was also observed in our study of nanoparticle synthesis. Many studies have shown that nanoparticles are made of betalain with various metals, and their properties have been synthesised, although fewer studies have shown that nanoparticles made with silver nitrate were used to antimicrobial activity [38,39].

Feng QL et al., had reported that AgNPs will increase cell membrane permeability and subsequently penetrate into cells, free radicals produce oxidative stress in Reactive Oxygen Species (ROS) resulting in damage membrane and Deoxyribonucleic Acid (DNA) [39]. Elayarajah B et al., have compared the mode of action of silver ions with the DNA damaging systems of significant oral drugs like fluoroquinolone and nitroimidazoles [40]. Also, the synergistic effect of various types of secondary metabolites or compounds in plant and plant parts such as glycosides, saponins, tannins, flavanoids and alkaloids always explained its activity against some microbial species [41]. The phenomenon of green synthesised betalain extract demonstrating greater antimicrobial activity shall be attributed to the fact that the released Ag^+ along with the plant extract plays solid antimicrobial activity by interacting with the cell wall and cell membrane components of the bacteria, which is one of the vital mechanisms of toxicity of AgNPs [42].

The first step in creating biofilms is to attach the bacteria to any surface. Many factors can affect bacterial adherence, including the growing environment, bacterial reliability, and material surface properties. The effect of AgNPs on bacterial adhesions were explored in this work. AgNPs have also been shown in several studies to reduce swarming ability and biofilm formation, leading in decreased pathogenicity [43-45]. Plant-synthesised AgNPs offer several advantages over non synthesised AgNPs, because of their ability to act as capping and stabilising agents of metabolites, enhanced antibacterial activity.

The antimicrobial activity of nanoparticles depends on the size-smaller particles interact more easily with the cell surface and attach more easily to the cytoplasm due to less spatial barrier. In addition, small nanoparticles provide a large area to interact with microorganisms or biological components, so they are highly effective. The small size (17.4 - 45.3 nm) of AgNPs in this study was another contributing factor to its antibacterial and antibiofilm effect. Similarly, Singh P et al., used 15 nm size AgNPs to control the biofilm formation [44]. A previous study also reported the antibiofilm potential of AgNPs against human pathogens. They record that nanoparticles with a particle size of less than 100 nm have excessive antibiofilm activity [45]. In current report, significant inhibition in biofilm formation was observed at 7.5 mg/mL of AgNPs. In 2015, Goswami SR et al., also investigated biofilm inhibition with AgNPs, and discovered that 15 mg/mL of AgNPs suppressed biofilm development in *S. aureus* by 89% and 75% in *E. coli*. [46]. Based on the current findings AgNPs were found to have an effect on biofilm formation.

Limitation(s)

Present findings had some limitations. The antimicrobial activity of these plant extracts seems to be significant however, current knowledge is mainly based on in-vitro studies, so its applicability in the clinical setting is still unknown. Bioactive compounds have been largely unknown in terms of their structure activity relationships and mechanisms of action until recently. As the structure pharmacology of this betalains is still unknown, further discovery of the pharmacology of these compounds may lead to standardisation of therapeutic regimens.

CONCLUSION(S)

It can be concluded that betalanin has many beneficial therapeutic properties such as antimicrobial activity and control of biofilm formation. The presence of the beet pigment of betalanin identified in this study and its combined function may be beneficial in controlling diseases. Especially prepared nanoparticles can be used as bactericidal agents, due to this application in the medical field; this research is encouraging to increase research on nanoparticles. Hence, further studies should be conducted in isolating and purifying the phytochemicals obtained in the study as an active ingredient and conducting a clinical study in the human population.

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