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ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF BRASSICA JUNCEA EXTRACTS AND SILVER NANOPARTICLES: A SYNERGISTIC APPROACH AGAINST ESCHERICHIA COLI

Aayushi Mishra*, A Aamalraj, Somendra Kumar, Suneel Sharma
Shri Ramnath Singh Institute of Pharmaceutical Science & Technology, Gwalior (M.P.)

**Corresponding Author:* *Aayushi Mishra, M.Pharma Research Scholar, Shri Ramnath Singh Institute of Pharmaceutical Science & Technology, Gwalior (M.P.)*

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ABSTRACT

Brassica juncea, a plant known for its anticancer and antioxidant characteristics, is rich in glucosinolates, flavonoids, carotenoids, and phenolic acids. AEBJ exhibited a high concentration of total flavonoids, measuring 201.09 ± 0.232 mg/g in terms of quercetin equivalents. However, it had a relatively lower total phenolic content, measuring 49.43 ± 1.114 mg/g in terms of tannic acid equivalents. The structural characterisation of silver nanoparticles was performed using UV-Visible spectroscopy, followed by the study of these nanoparticles utilising surface plasmon resonance (SPR) measurements. The interaction between silver ions and extracts of Brassica juncea was seen, and the presence of AgNPs was verified through the detection of a visible peak at 425 nm. The Silver Nanoparticle (BJ 1) effectively suppressed the generation of DPPH radicals within the concentration range of 10-100 µg/ml. The linear regression coefficients for this inhibition were calculated to be 0.9869 and 0.9488, respectively. The IC50 values for Silver Nanoparticle (BJ 1) and BHT were 45.413 and 50.173, respectively. Silver Nanoparticle (BJ 1) exhibited a decrease in the conversion of ferricyanide to ferrocyanide as the concentration increased from 50-250 µg/ml. This was accompanied by an increase in the absorption of green light at a wavelength of 700 nm. The investigation into the antibacterial activity of silver nanoparticles (BJ 1) and Brassica juncea extracts against Escherichia coli has demonstrated substantial potential as antimicrobial agents. The findings indicate that both drugs demonstrate inhibitory effects on the growth of E. coli, with the concentration levels playing a substantial influence. This implies a mutually beneficial interaction between the two drugs, which could potentially amplify their efficacy in fighting bacterial infections. Greater doses of Brassica juncea extract exhibited bigger areas of inhibition, so validating its bioactive activities against pathogenic microorganisms. Furthermore, the Silver nanoparticle (BJ 1) had a potent antibacterial impact as a result of their capability to disturb bacterial cell membranes and impede metabolic activities. The combination of these two medicines has the potential to enhance therapy efficacy in clinical settings. Subsequent investigations should delve into the mechanisms underlying their antibacterial properties and evaluate their safety profiles for future medicinal uses.

KEYWORDS: *Brassica juncea; Silver Nanoparticles; Antioxidant Activity; DPPH; Reducing Power Assay; Antimicrobial Activity; Escherichia coli*

1. INTRODUCTION

Plants contain a variety of secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, which have antibacterial properties. Microbial resistance to existing antimicrobial drugs has led to the development of new compounds that can impede microorganism growth. Phytochemicals with antioxidant capabilities may reduce cancer occurrence and mortality rates in various human populations. Medicinal plants serve as the primary reservoir for medications with antioxidant and antibacterial properties [1]. These agents regulate the growth of dangerous bacteria, specifically E.coli and Salmionella typii, and regulate the activity of harmful free radicals. Medicinal herbs are the primary reservoir for antioxidants and antibacterial medications, playing a crucial role in drug discovery. Although the antioxidant and antibacterial activity of many plants have been assessed, there is always a need to discover new medications. Brassica juncea and medicago sativa were chosen for studies on antioxidant and antibacterial activities. Shore roxhburghii, a tropical plant with numerous phenolic components, was also analyzed for its antioxidant and antibacterial properties [2]. Brassica juncea is known for its rich content of glucosinolates and phenolic compounds, which play a crucial role in the reduction of silver and gold ions. The extracts from this plant not only facilitate the reduction process but also provide capping agents that prevent agglomeration of the formed nanoparticles. The utilization of Brassica juncea for nanoparticle synthesis not only highlights the potential for sustainable

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practices in nanotechnology but also paves the way for further research into optimizing conditions for enhanced yield and functionality [3, 4]. Recent studies have focused on plant-based synthesis methods as eco-friendly alternatives to conventional chemical approaches. Brassica juncea (mustard) have shown promise in the green synthesis of nanoparticles. These plants contain phytochemicals that can reduce metal ions to form nanoparticles while simultaneously stabilizing them.

2. MATERIALS AND METHODS

2.1 Collection of the Plant Materials

The Stem bark of Brassica juncea was obtained from Villages near by Gwalior (Madhya Pradesh) and was authenticated by the botany department of Institute of Professional Studies, Gwalior, Madhya Pradesh.

2.2 Preparation of the Extracts

The stem barks of Brassica juncea was dried in the shade and then ground into a powder using a miller. 50 gm of the powdered materials were placed in the thimble and introduced into a double bypass Soxhlet apparatus. This apparatus was connected to two distillation flasks through inverted Y-shaped joints. The materials were then extracted with 500 ml Distilled water. The solvent was evaporated, resulting in the aqueous extract of stem barks of Brassica juncea (AEBJ).

2.3 Estimation of the Phytochemical Constituents

2.3.1 Qualitative estimation of Phytoconstituents

These tested were conducted for the estimation of the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils using standard procedures in extract [5].

2.3.2 Quantitative Estimation of Phytoconstituents

2.3.2.1 Total Phenolic Content

The extract's total phenolic content was determined using spectrometry [6]. Folin-Ciocalteu's reagent was added to a sample, tannic acid (10-100 µg/ml), sodium carbonate (75 g/l), and distilled water. The mixture was stirred for 2 hours at room temperature, and then centrifuged at 2000 rpm for 5 minutes. The absorbance was read at 760 nm, and a standard curve was obtained using different tannic acid concentrations. Results were expressed as mg of tannic acid equivalents per gram of extract.

2.3.2.2 Total Flavonoids Content

The aluminum chloride colorimetric assay measures the total flavonoid content of extracts [7]. A sample or standard solution of quercetin is added to a 10 ml volumetric flask containing distilled water. Afterward, 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH are added. The solution is mixed, and absorbance is measured at 510 nm. The total flavonoid content is expressed as milligrams of quercetin equivalents per gram of extract.

2.4 Synthesis of Silver Nanoparticles using Brassica Juncea Stem extract

A 1mM $AgNO_3$ solution was prepared by dissolving 0.085gms $AgNO_3$ in 500 ml distilled water and stored in an amber colored bottle. In an Erlenmeyer flask, 75 mL of Brassica juncea stem extract of different concentration (25, 50, 100, 150, 200 µg/ml) was added to the solution for bio reduction. The reaction mixture was stirred at 200 rpm until the solution turned from yellow to dark brown, indicating the formation of AgNPs. The reduced solution was centrifuged at 5000 rpm for 30 minutes to obtain a clear supernatant, which was then discarded and the particles were centrifuged with water to obtain pure nanoparticles. Various formulations of silver nanoparticles (BJ1, BJ 2, BJ 3, BJ 4, and BJ 5) were prepared using Brassica juncea extract of different concentration (25, 50, 100, 150, 200 µg/ml) and 1 mM silver nitrate solution. The reduction of elemental Ag to AgO was confirmed by the color change from colorless to brownish-yellow, indicating the encapsulation of aqueous mustard extract into silver Nanoparticles [8].

2.5 Characterization of Silver Nanoparticles using UV-Visible Spectroscopy

The synthesized AgNPs (solution of 1 mg/mL in distilled water as a dispersive medium) were monitored by employing the periodic scans of the optical absorbance between 300 and 700 nm with a double-beam UV-visible spectrophotometer (Carry 100 with tungsten halogen light sources) at room temperature to investigate the reduction of silver ions by the extract. Distilled water was used as blank [9].

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2.6 Antioxidant activity of Silver Nanoparticles

2.6.1 DPPH Radical Scavenging Activity

The antioxidant activities of the silver nanoparticles have been studied through the evaluation of the free radical-scavenging effect on the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical. Various concentrations (20, 40, 60, 80 and 100 µg/mL) of silver nanoparticles (1.0 mL of 0.1mM DPPH) were mixed with 3.0 mL of methanolic solution containing DPPH radical (6x10 -5 mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by recording the 65 absorbance at 517 nm. The radical scavenging activity (RSA) was calculated as the percentage of DPPH discolouration [10].

2.6.2 Reducing power assay

The reducing power of the extract was determined as per previously described method. Different concentrations of Silver Nanoparticle (BJ 1) (50-250 µg/ml) were prepared in distilled water. Each concentration (0.5 ml) was mixed with phosphate buffer (1.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (1.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (1.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (1.5 ml) was diluted with distilled water (1.5 ml). Finally, FeCl3 (300µl, 0.1%) was added and again centrifuged at 3000 rpm for 5 min. and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as standard antioxidant. The experiment was performed in triplicate [11].

2.7 Evaluation of Anti-Microbial Activity

2.7.1 Preparation of Discs

Filter paper discs of 6mm diameter are prepared and autoclaved in a clean and dry Petri plate. They are soaked in plant extracts for 6 hours, then shade dried. The concentrations are recorded as 0.1 grams per disc. The discs are then spread on cultured Petri plates, and those immersed in Butanol, Benzene, and distilled water are used as controls [12].

2.7.2 Anti-Microbial Activity Assay

The antibacterial activity was tested using the Kirby-Bauer method. This method involves placing paper discs impregnated with antibiotics onto a lawn of bacteria on an agar plate. The antibiotics diffuse into the agar, creating a gradient of concentration that inhibits bacterial growth. The size of the zone of inhibition around each disc is then measured to determine the effectiveness of the antibiotic against the tested bacteria. MHA plates were inoculated with Bacterial culture of Escherichia coli, and discs containing Silver Nanoparticle (BJ 1) and different concentration of aqueous extract of stem of Brassica juncea (AEBJ) (6.25, 12.5, 25, 50 and 100 µg/ml)were placed. 10% of the sample was diluted to achieve the required amount. A vehicle control was loaded with solvent alone, while a Ciprofloxacin disc (10µg) was used as a positive control. The plates were incubated at 37°C for 24 hours, and clear zones around the discs were measured and recorded [13].

2.8 Statistical analysis

All the necessary data were expressed as mean ± SD. Analysis of variance was performed by the ANOVA procedures. The significance of results was analyzed using one-way ANOVA. $P<0.05$ is considered as significant difference.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Phytochemical Constituents

3.1.1 Qualitative Estimation of Phytochemical Constituents

Brassica juncea contains glucosinolates, flavonoids, carotenoids, and phenolic acids, which have anticancer properties and antioxidant properties. Flavonoids and carotenoids protect cellular components from oxidative damage and have anti-inflammatory effects, potentially reducing chronic diseases like cardiovascular conditions. Coumarins have anticoagulant properties, potentially preventing thrombosis-related disorders.

3.1.2 Quantitative Estimation of Phytochemical Constituents

The quantitative estimation of phytoconstituents viz. total flavonoids and total phenolics and saponins revealed that AEBJ was found rich in total flavonoids with 201.09 ± 0.232 quercetin equivalents mg/g of AEBJ while less amount of total phenolic content with 49.43 ± 1.114 tannic acid equivalents mg/g of AEBJ.

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3.2 Characterization of Silver Nanoparticles

3.2.1 UV-Visible Spectroscopy

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of silver Nanoparticles. The bio-transformed products were simultaneously characterized by UV-Visible spectroscopy measurements performed at different time intervals to study the change in light absorption profile of the solution and increase in intensity (Figure 1). The progress of the reaction between silver ions and extracts of Brassica juncea was monitored by recording the surface plasmon resonance (SPR) as a function of time. By employing the variable concentration (25, 50, 100, 150, 200 mg/ml) of the extract with silver nitrate (1.0 mM), the effect of concentration of the extract on the rate of bioreduction was studied. Since, this wavelength falls within the prescribed range, confirming the formation of AgNPs. Formulation BJ 1 showed the clear peak at 425 nm, so we selected the formulation BJ 1 for further studies.

3.2.2 Antioxidant activity of Silver Nanoparticles

3.2.2.1 DPPH Radical Scavenging Activity

The Silver Nanoparticle (BJ 1) in concentration range of 10-100 µg/ml inhibited DPPH radical formation as indicated by concentration dependent decrease in the purple colour of the solution. Similar effect was obtained with standard antioxidant- BHT in the concentration range of 10-100 µg/ml. In linear regression analysis of concentration versus percent DPPH inhibition was carried out. The linear regression coefficient of Silver Nanoparticle (BJ 1) and BHT were 0.9869 and 0.9488, respectively, suggesting that the DPPH scavenging was concentration dependent. The IC_{50} value of Silver Nanoparticle (BJ 1) and BHT, obtained from regression analysis, were 45.413 and 50.173, respectively (Table 1).

3.2.2.2 Reducing Power Assay

Silver Nanoparticle (BJ 1) in the concentration range of 50-250 µg/ml showed concentration related reduction of ferricyanide to ferrocyanide as indicated by increase in the green colour absorbance measured at 700 nm. Similar effect was also observed with standard antioxidant, ascorbic acid in the concentration range of 50-250 µg/ml. A concentration verses absorbance graph comparing ascorbic acid and Silver Nanoparticle (BJ 1) were plotted and depicted in Figure 2.

3.3 Effect of Silver Nanoparticle (BJ 1) and different concentration of aqueous extract of stem of Brassica juncea (AEBJ) on zone inhibition assay for Anti-microbial activity

In this experiment, effect of Silver Nanoparticle (BJ 1) and different concentration of aqueous extract of stem of Brassica juncea (AEBJ) (6.25, 12.5, 25, 50 and 100 µg/ml) was tested against Escherichia coli. The results showed that Silver Nanoparticle (BJ 1) was exhibited significant antimicrobial activity against E.coli. Also, zone inhibition effect of Silver Nanoparticle (BJ 1) against E.coli (Figure 3). The findings suggest that AEUD and AEXS have the potential to be used as natural alternatives to synthetic antibiotics in the treatment of microbial infections.

4. CONCLUSIONS

Brassica juncea, a plant with anticancer and antioxidant properties, contains glucosinolates, flavonoids, carotenoids, and phenolic acids. These compounds protect cellular components from oxidative damage and have anti-inflammatory effects, potentially reducing chronic diseases like cardiovascular conditions. Coumarins have anticoagulant properties, potentially preventing thrombosis-related disorders. AEBJ, rich in total flavonoids, was found to have less total phenolic content. UV-Visible spectroscopy was used to structurally characterize silver nanoparticles, which were studied using surface plasmon resonance measurements. The formation of AgNPs was confirmed by the visible peak at 425 nm. Silver Nanoparticle (BJ 1) inhibited DPPH radical formation in concentrations of 10-100 µg/ml, with IC50 values of 45.413 and 50.173, respectively. The study on silver nanoparticle (BJ 1) and Brassica juncea extracts' antimicrobial activity against Escherichia coli revealed significant potential as antimicrobial agents. Both substances exhibit inhibitory effects on E. coli growth, with concentration levels playing a significant role. Combining these two agents could lead to more effective treatment strategies in clinical settings. Future research should explore the mechanisms of their antibacterial action and assess their safety profiles for potential therapeutic applications.

5. CONFLICT OF INTEREST

None

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FIGURES AND TABLES

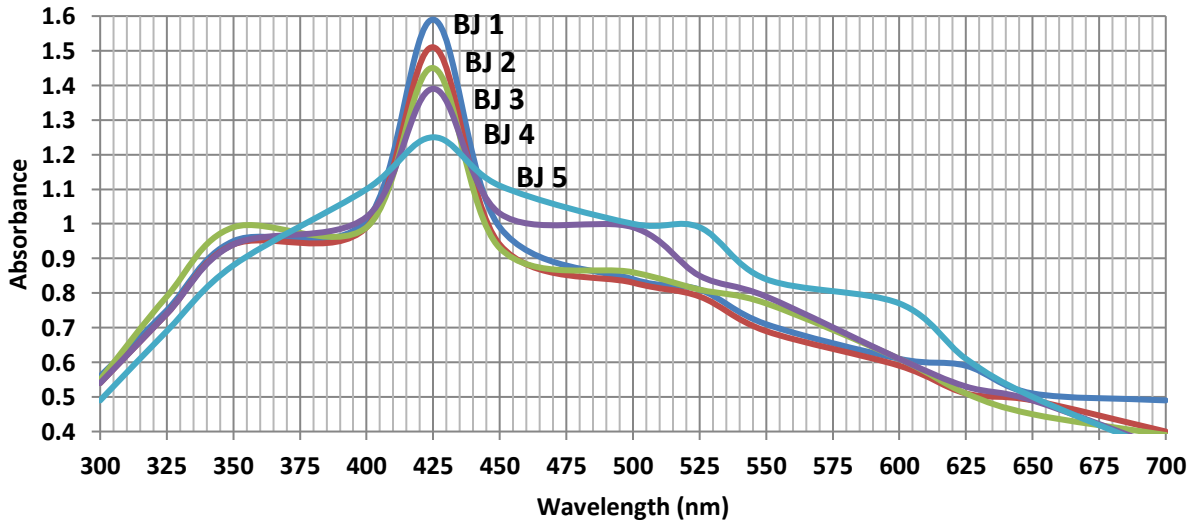


Figure 1: Characterization of Silver Nanoparticles (BJ1, BJ 2, BJ 3, BJ 4, and BJ 5) prepared using Brassica juncea extract of different concentration (25, 50, 100, 150, 200 µg/ml) using UV-Visible Spectroscopy

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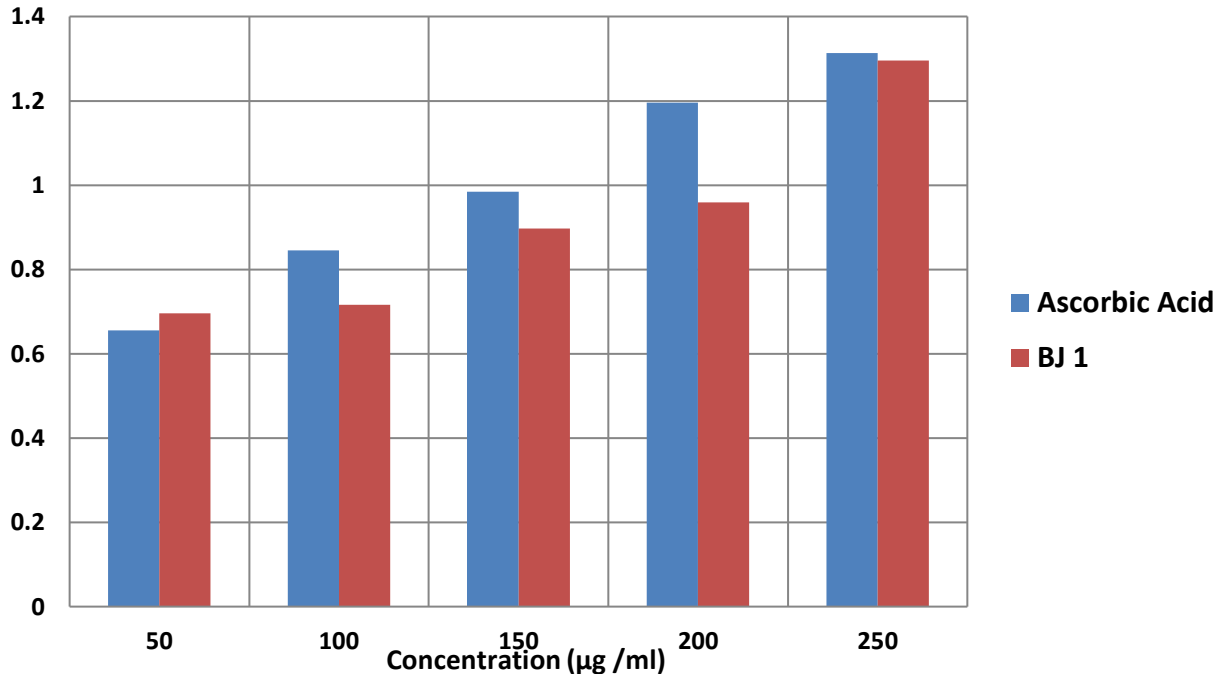


Figure 2: A concentration verses absorbance graph comparing ascorbic acid and Silver Nanoparticle (BJ 1) showing Reducing Power Assay

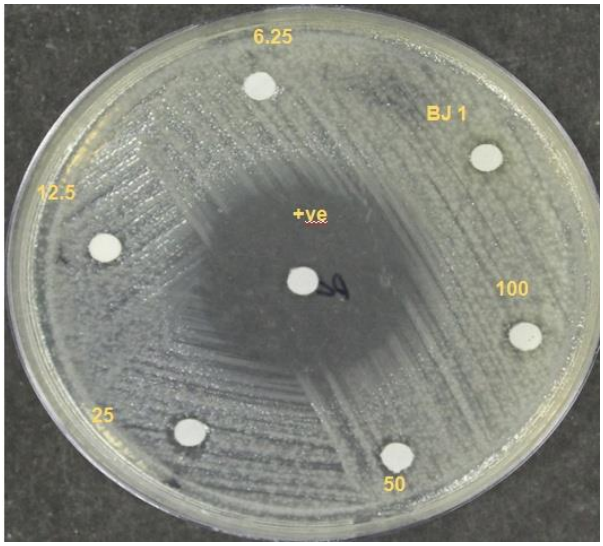


Figure 3: Anti-microbial activity of Silver Nanoparticle (BJ 1) and different concentration of aqueous extract of stem of Brassica juncea (AEBJ) against the Escherichia coli

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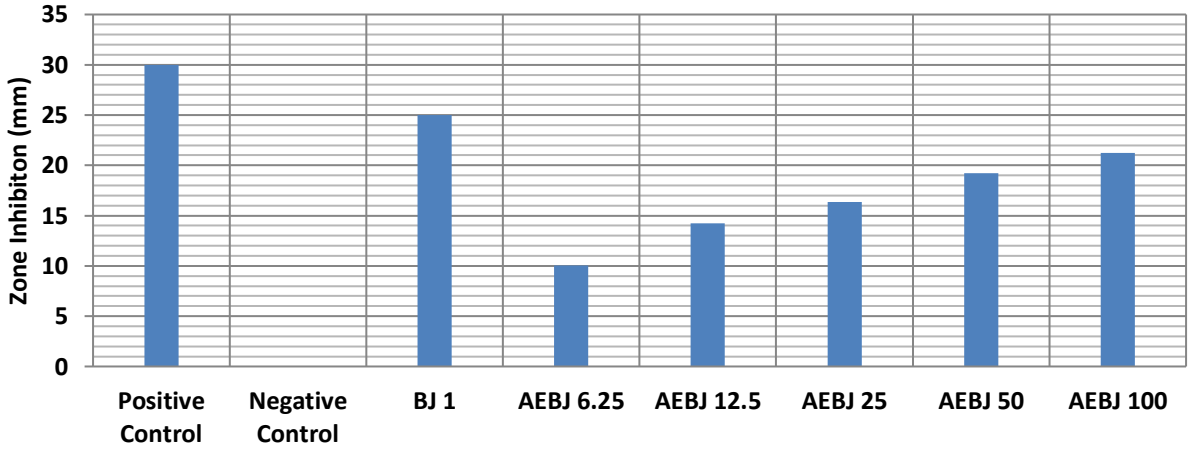


Figure 4: Effect of Silver Nanoparticle (BJ 1) and different concentration of aqueous extract of stem of Brassica juncea (AEBJ) on zone inhibition assay for Anti-microbial activity against the Escherichia coli.

Table 1: Effect on DPPH Radical Scavenging Activity

Concentration (µg/ml)		% Inhibition	IC_{50} Value
BHT	10	13.311 ± 0.397	50.173 µg/ml
	20	25.706 ± 0.529	
	40	47.305 ± 0.496	
	60	65.163 ± 0.636	
	80	75.064 ± 0.223	
	100	80.271 ± 0.257	
Silver Nanoparticle (BJ 1)	10	13.343 ± 0.397	45.413 µg/ml
	20	26.514 ± 0.563	
	40	47.991 ± 1.028	
	60	65.176 ± 0.636	
	80	74.740 ± 0.532	
	100	81.279 ± 1.150	

Values are mean± SEM; n=3; IC_{50}= 50% Inhibitory concentration