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SYNTHESIS AND CHARACTERIZATION OF TERMINALIA ARJUNA BARK EXTRACT-BASED SILVER NANOPARTICLES: PROCESS PARAMETERS OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY AND EVALUATION OF ANTIBACTERIAL ACTIVITY

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Nanoparticles (NPs) synthesized by the green synthesis approach have appeared as an environmentally friendly and sustainable strategy in the past decade. In this process, the bark extract of *Terminalia arjuna* (*T. arjuna*) functions as a reducing and capping agent for synthesizing silver nanoparticles (AgNPs). Different bioactive compounds, such as saponins, tannins, and flavonoids from bark extract, play an essential role in nanoparticle synthesis and stabilization. The appearance of a dark brown color in the solution visually confirms the nanoparticle formation. Furthermore, an absorbance peak at 410 nm wavelength confirms the bio-synthesis of AgNPs. Moreover, process parameters (temperature, stirring time, and silver nitrate concentration) for nanoparticle synthesis were statistically optimized by using the response surface methodology (RSM) tool. The maximum synthesis of nanoparticles was achieved at 5 mM silver nitrate, 80° C temperature, and 40 minutes of stirring time. The spherical structure of AgNPs, with a size range of 10-120 nm and an average particle size of 36 nm, was discovered from transmission electron microscopy (TEM). Also, the X-ray diffraction (XRD) technique indicated the crystalline nature of synthesized AgNPs. Moreover, the antibacterial potential of silver nanoparticles was tested against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), which exhibit significant inhibition zones and advocate using AgNPs against antibiotic-resistant strains. This study reveals the capability of *T. arjuna* bark extracts in the biogenic synthesis of NPs with high antibacterial potential, which can be employed in different biomedical applications.

Key words: green synthesis; silver nanoparticles; terminalia arjuna; response surface methodology; antibacterial activity

INTRODUCTION

Nanotechnology has attained notable consideration in the past few decades, especially in the research of metallic nanoparticles, as they exhibit greater physiochemical and biological properties than their bulk analogs [1]. Amongst metallic nanoparticles, silver nanoparticles (AgNPs) are extensively explored in various disciplines, such as biomedical, electronics, food, catalysis, imaging, and sensing [2]. There are numerous conventional approaches for synthesizing silver nanoparticles, but these approaches are energy extensive and use toxic chemicals and reagents that are hazardous to the environment. Due to this limitation, there is a need for a process that is more sustainable, greener, and safer [3].

Green synthesis is a process in which a biological source (plants, microorganisms) is used to synthesize nanoparticles. Green synthesis uses plant and microorganism metabolites as stabilizing,

reducing, and capping agents [4]. In the study, *Terminalia arjuna* (*T. arjuna*) bark extract is used as it contains biomolecules, for instance, phenols, tannins, flavonoids, and alkaloids, which facilitate the silver ion reduction and stabilize the formed NP [5]. Furthermore, Response Surface Methodology (RSM) is also used to optimize essential process conditions such as temperature, silver nitrate concentration, and stirring time [6]. Furthermore, the characterization of *T. arjuna* NPs was done by utilizing TEM, XRD, and UV-visible spectroscopy. Moreover, the antibacterial potential of synthesized NPs was checked against *S. aureus* and *E. coli*.

The aim of the study was to examine a green, sustainable, and eco-friendly method for producing AgNPs using *T. arjuna* bark extract, exploiting its biomolecules as a stabilizing, capping, and reducing agent. The process parameters for synthesis were also optimized with the help of Response Surface

Table 1 – Design strategy and factor boundaries utilized for process parameter optimization (Central Composite Design) for *T. arjuna* nanoparticles

Process parameters	Level	
	(-1) low	(+) high
Silver nitrate concentration (mM)	1	5
Temperature (°C)	30	80
Stirring time (min.)	4	80

Table 2 – CCD for optimizing process parameters [factors: (A) silver nitrate conc., (B) temperature, and (C) stirring time] for *T. arjuna* AgNPs

Run	Silver nitrate concentration (mM)	Temperature (°C)	Stirring time (min.)	Weight of nanoparticles (mg/ml)
1	3	55	26.3641	29.5
2	6.36359	55	60	40.9
3	5	80	40	33
4	5	30	80	35.8
5	5	30	40	24.3
6	1	80	80	10.3
7	3	55	60	21.4
8	3	55	60	20.8
9	1	30	80	8.2
10	3	55	93.6359	24.3
11	3	55	60	19.7
12	1	30	40	7.7
13	3	12.9552	60	24
14	3	55	60	32
15	3	55	60	36
16	3	55	60	34
17	1	80	40	30
18	0.363586	55	60	16
19	5	80	80	20
20	3	97.0448	60	22

Methodology (RSM) to ensure maximum yield for AgNPs. Lastly, the antibacterial activity of AgNPs was evaluated against selected bacteria to discover the potential of AgNPs as an alternative to antibiotics.

MATERIALS AND METHODS

Materials required

Silver nitrate (AgNO₃) (99.9%, ACS, 169.87mw) and sodium hydroxide (NaOH) (97%, ACS, 40mw) were procured from Sisco Research Laboratories, India. *T. arjuna* bark was purchased from the local market; it was thoroughly washed, dried, and ground into fine powder. Deionized water was prepared in the lab itself. *S. aureus* and *E. coli* strains were attained from the Department of Life Sciences, Sharda University (India). Ethanol (99.9%, ACS) was acquired from a local supplier.

T. arjuna bark extract preparation

The extract was made by adding 10 g of *T. arjuna* bark powder to 100 ml of deionized water, heated for 15 min, and let to cool at room temperature. The solution was filtered, and the extract was stored at 4 °C until further use.

Synthesis of *T. arjuna* bark extract mediated AgNPs

For the formation of AgNPs, different concentrations of AgNO₃ solutions were taken, and the concentration with the most favorable was selected. To synthesize AgNPs, 10 ml of bark extract was added dropwise to 1mM of 100 ml of AgNO₃ solution. 1M NaOH solution was used to maintain the pH at 9. The solution was then heated for 60 minutes at 60 °C to enhance the production of nanoparticles. A brownish pellet was obtained after centrifuging at 10000 rpm,

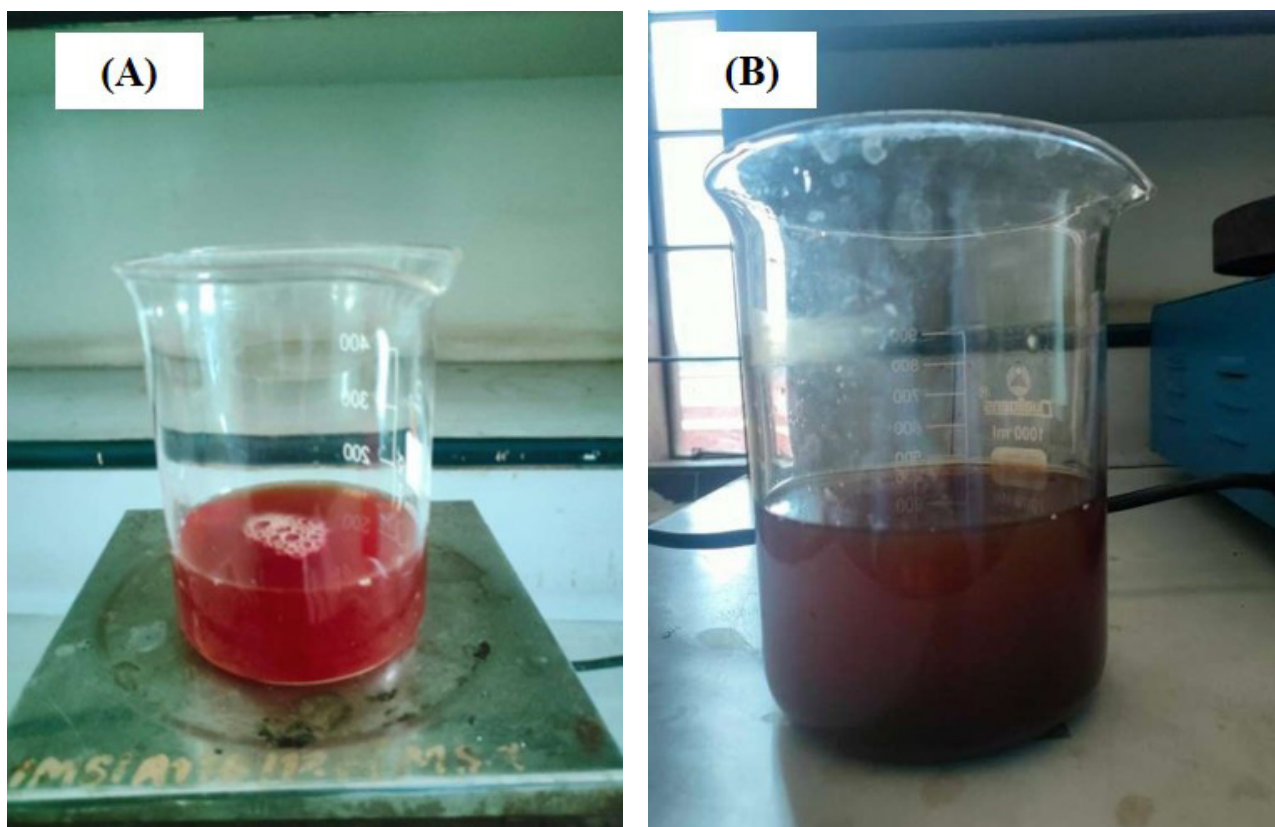


Figure 1 – *T. arjuna* extract and salt mixture before NPs formation (a) and extract and salt mixture after NPs formation (b)

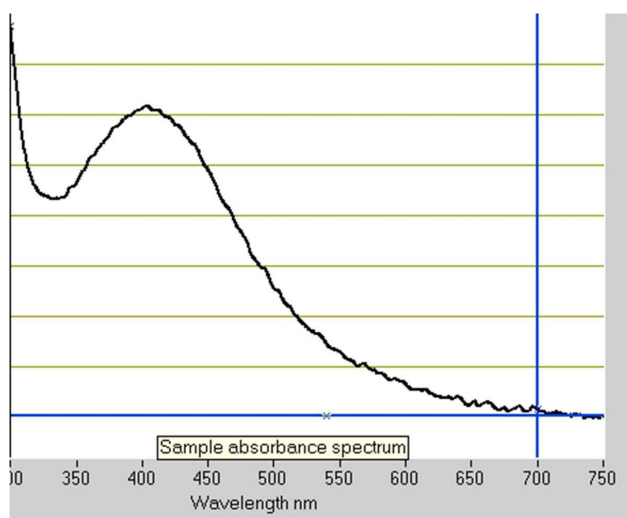


Figure 2 – UV-Vis spectrum *T. arjuna* extract mediated synthesized AgNPs

washed with 80% ethanol, and dried at room temperature.

Characterization

In the present study, UV-visible spectroscopy was used to find the maximum peak absorbance of AgNPs. The scan range was set at 200nm – 800nm wavelength. TEM was utilized to study the structure of AgNPs at high magnifications. Finally, the crystalline nature of AgNPs was determined by employing XRD.

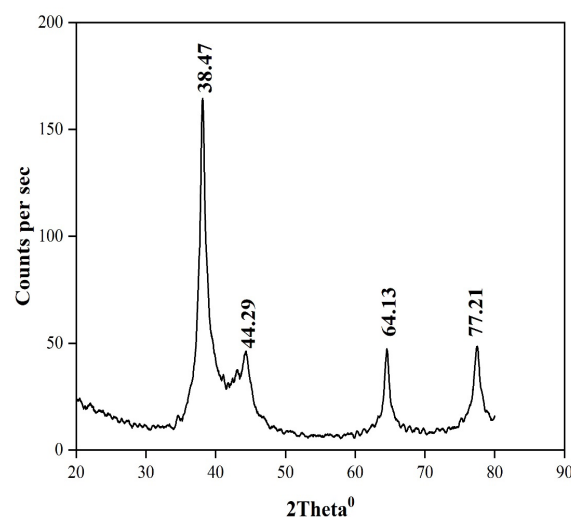


Figure 3 – XRD patterns of the synthesized silver nanoparticles

Optimization of process parameters

The central composite design (CCD) under the response surface methodology (RSM) approach was utilized to optimize process parameters. The factors were taken as silver nitrate concentration (A), temperature (B), and (C) stirring time. Table 1 depicts all three parameters of *T. arjuna* – mediated synthesis of nanoparticles at two levels, high (+1) and low (-1). A series of 20 experiments were car-

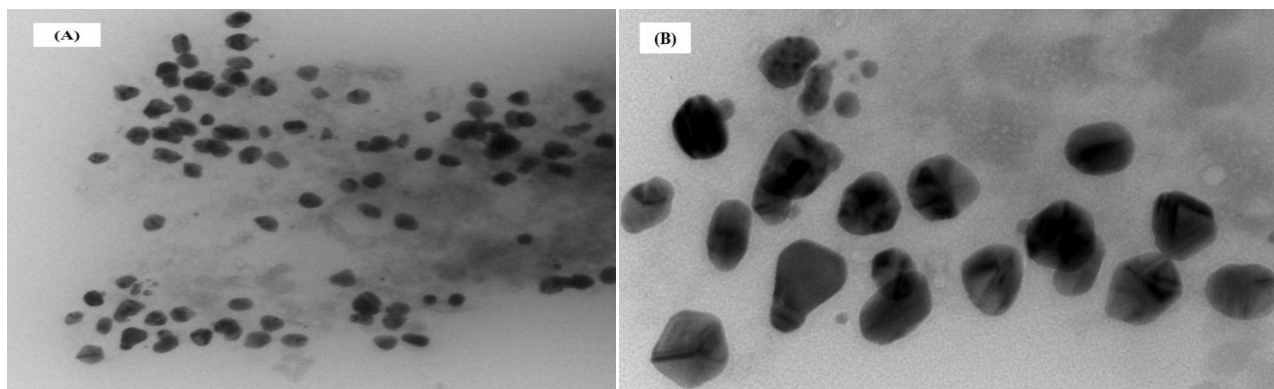


Figure 4 – TEM image of the AgNPs at 25KX (a) and 40KX magnifications (b)

ried out, and the weight (mg) of nanoparticles produced as a response (R1) was recorded. Table 2 shows the central Composite Design for optimizing process parameters. Statistical analysis, model fitting, and 3D response surface plots were generated using Design Expert (version 13).

Assessment of the antibacterial potential

The antibacterial potential of produced AgNPs was studied using the agar well diffusion method. Firstly, the 100ul culture was spread on nutrient agar media containing petri plates. Four bores of 6mm were bored on agar plates with the help of a borer. Distilled water was filled in well 1 as a blank, the wells marked as 2 were filled with 50mg/ml amoxicillin, and 3 and 4 were filled with synthesized silver nanoparticles with concentrations of 50 mg/mL and 150 mg/mL. Plates were incubated at 37°C, and the size of the zone of inhibition (mm) was determined.

RESULTS AND DISCUSSIONS

Synthesis

A distinct color shift was observed from reddish to brownish (Figure 1 a, b) because of the reduction of the metal ions contained in the precursor salt, indicating the creation of nanoparticles. The surface plasmon (SPR) peak was observed at 410 nm in the UV-visible spectroscopy analysis (Figure 2).

Characterization of synthesized AgNPs

X-ray diffraction (XRD)

An X-ray diffraction (XRD) graph was used to find the crystalline nature of the nanoparticles. The noticeable peaks at 38.47°, 44.29°, 64.13°, and 77.21° (Figure 3) correspond to the pure silver planes, with h, k, l values 111, 200, 220, and 31. This confirms that the AgNPs are crystalline and have a silver composition [7].

Transmission electron microscopy (TEM)

As revealed by TEM, AgNPs were spherical (Figure 4 a, b), having a size distribution of 10-120 nm and a mean particle size of 36 nm. Minor agglomeration was also seen due to the effective capping and stabilization of phytochemicals in the *T. arjuna* extract [8].

Optimization

The Optimization of process parameters was done by utilizing central composite design (CCD). The p-value ($p < 0.05$) indicates that the three variables (silver nitrate concentration, temperature, and stirring time) strongly influenced nanoparticle synthesis. Table 3 illustrates the fit summary of different models; Table 4 shows ANOVA for the 2FI model. 3D-surface graphs of varying process parameters were revealed by Figure 5 a, b, c.

The optimal conditions for synthesizing AgNPs were silver nitrate concentration of 5 mM, temperature 80 °C, and stirring time of 40 minutes. The nanoparticle's experimental weight was 22mg/ml after synthesizing AgNPs under optimized conditions.

Antibacterial potential

Significant antibacterial activity (figure 6 a, b) was observed against both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). Inhibition zones of 16 mm and 10 mm were seen for *S. aureus* and *E. coli* at 150 mg/ml of AgNPs. The mechanism involved in antibacterial activity is the disruption of bacterial cell membranes and generating reactive oxygen species (ROS) [9]. Table 5 shows the observed inhibition zone against *E. coli* and *S. aureus*.

DISCUSSION

This current study portrays a green, sustainable, and safer approach for synthesizing AgNPs using *T. arjuna* bark extracts. The SPR peaks around 410 nm, and the color shift from reddish to brown signifies the formation of *T. arjuna* AgNPs, with biomolecules present in the extract acting as reducing, capping, and stabilizing agents.

The optimization utilizing response surface methodology showed that the process parameters significantly influenced nanoparticle yield, with optimal parameters such as temperature 80 °C, AgNO₃ concentration of 5 Mm, and stirring time of 40 minutes.

Characterization techniques such as TEM and XRD disclosed that the AgNPs were spherical and averaging 10-120nm in size. Moreover, the synthesized AgNPs featured significant inhibition zones, indicating their potential use in biomedical applications.

Table 3 – Fit summary of models of optimization of *T. arjuna* AgNPs

Source	Sequential p-value	Lack of Fit P-value	Adjusted R ²	Predicted R ²	Remark
2FI	0.00422	0.8267	0.5758	0.237	Suggested
Linear	0.0159	0.5241	0.3665	0.1491	–
Cubic	0.9643	0.8394	0.5692	0.2841	–
Quadratic	0.4600	0.8394	0.5692	0.2841	–

Table 4 – ANOVA for 2FI model of *T. arjuna* AgNPs

Source	Sum of Squares	df	Mean Square	F value	P value	Remark
Model	1205.09	6	200.85	5.30	0.0058	Significant
Silver nitrate concentration	714.43	1	714.43	18.85	0.0008	–
Temperature	14.22	1	14.22	0.3752	0.5508	–
Stirring time	63.49	1	63.49	1.67	0.2181	–
BC	249.76	1	249.76	6.59	0.0234	–
AB	124.03	1	124.03	3.27	0.0927	–
AC	39.16	1	39.16	1.03	0.3280	–
Lack of fit	215.31	8	26.91	0.4850	0.8267	Not significant
Residual	492.80	13	37.91	–	–	–
Pure Error	277.49	5	55.50	–	–	–
Cor Total	1697.89	19	–	–	–	–

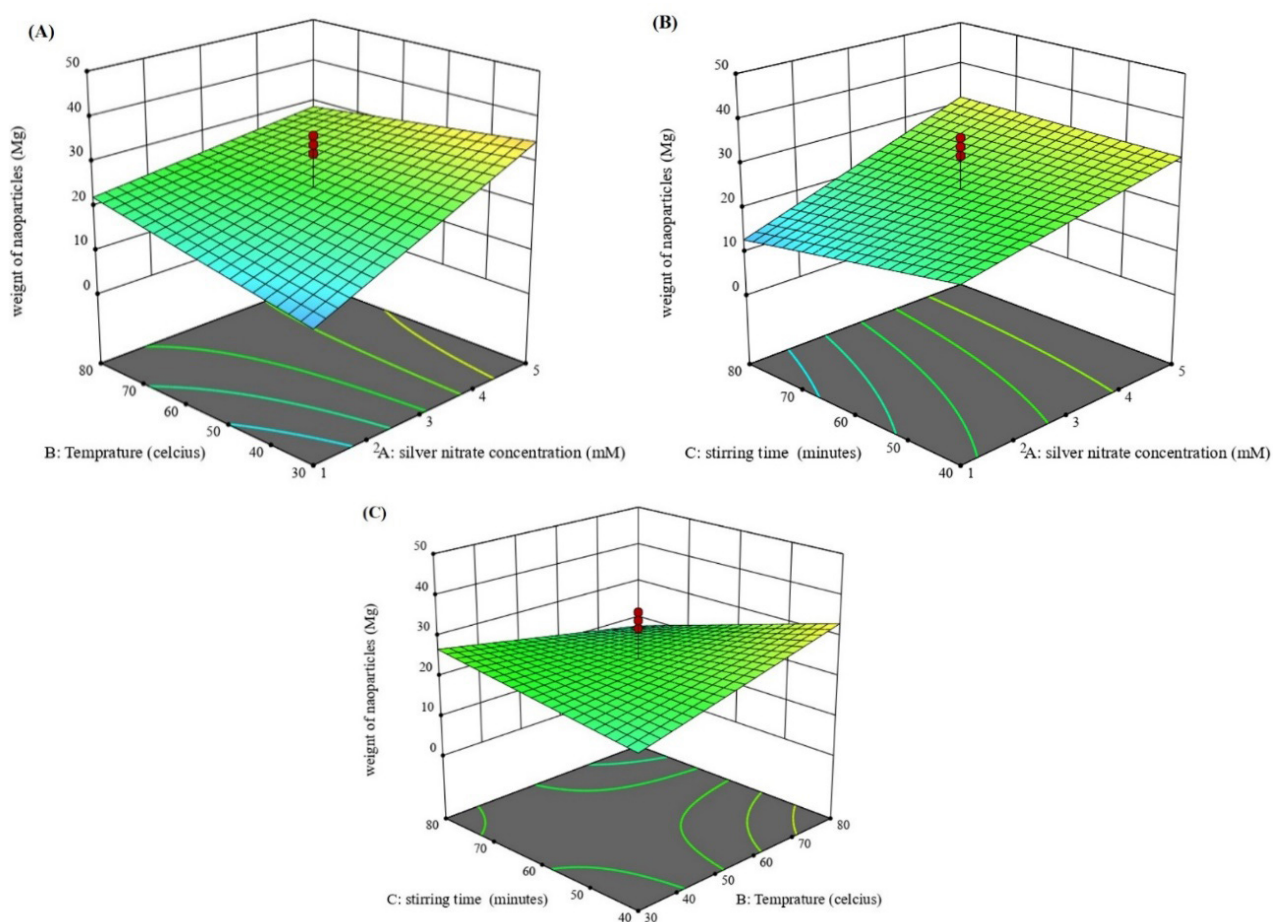


Figure 5 – Response surface plot for (a) weight of nanoparticles (mg/ml) vs. silver nitrate conc. vs. temperature, (b) weight of nanoparticles (mg/ml) vs. silver nitrate conc. vs. stirring time (c) weight of nanoparticles (mg/ml) vs. temperature vs. stirring time

Table 5 – Inhibition zone against *E. coli* and *S. aureus*

SNO	Bacteria	Amoxicillin (50 mg/ml)	AgNPs	
			100 mg/ml	150 mg/ml
1	<i>E. coli</i>	14 mm	9 mm	10 mm
2	<i>S. aureus</i>	12 mm	6 mm	16 mm

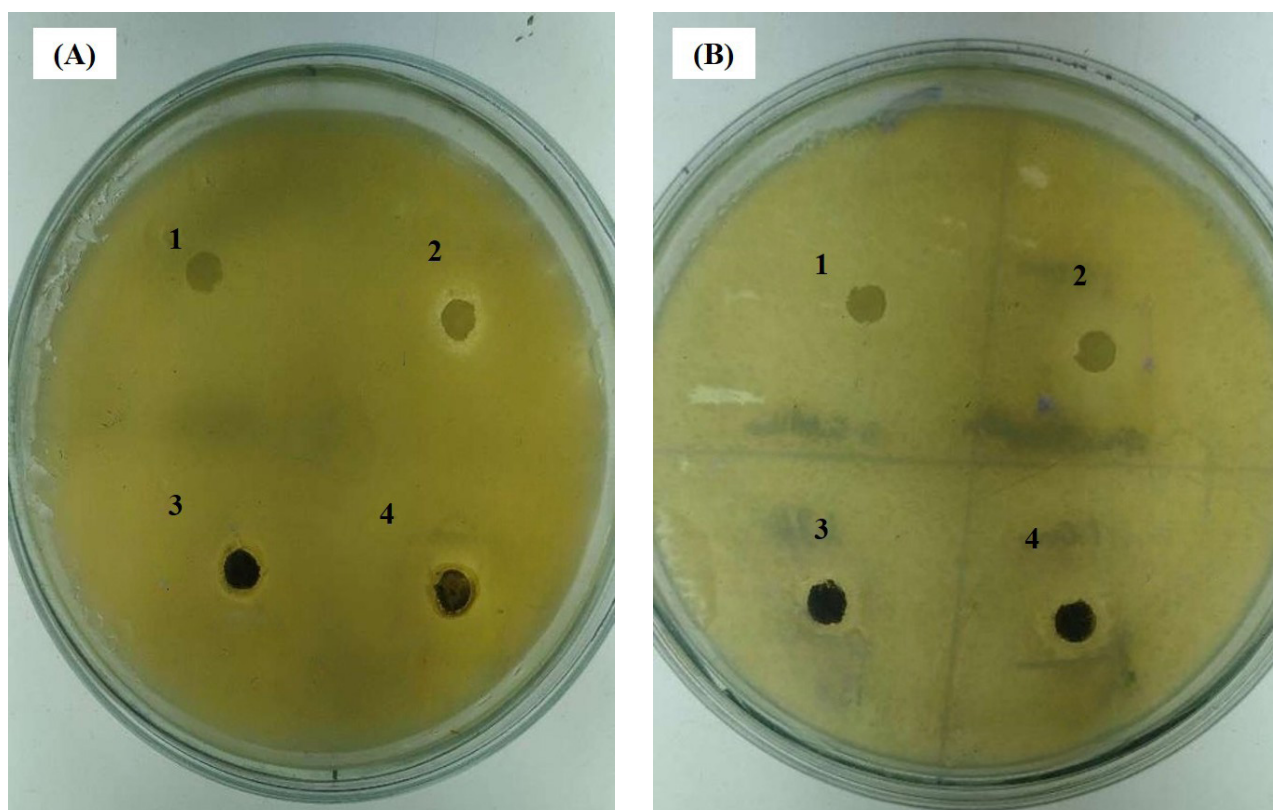


Figure 6 – Antibacterial potential of synthesized AgNPs against *E. coli* (a) and *S. aureus* (b)

CONCLUSION

This study successfully establishes a straightforward, greener, non-toxic, and environmentally friendly approach for synthesizing AgNPs using *T. arjuna* plant extract. This process used biomolecules present in the extract as reducing, capping, and stabilizing agents [10]. Optimization of process parameters has tremendously increased the yield of AgNPs. At the same time, characterization techniques such as TEM and XRD have conformed to forming spherical, crystalline, and stable nanoparticles [11].

Lastly, AgNPs showed antibacterial potential against Gram-positive and Gram-negative bacteria, showing their potential in biomedical and healthcare applications [12].

Authors' contributions:

P. Kaushik, A. K. Verma, R. Rupasmita, S. K. Mishra, M. Kumar – conceptualization, methodology.

A. K. Verma, R. Rupasmita – data curation, investigation.

P. Kaushik, S. K. Mishra, M. Kumar – formal analysis, software, visualization.

R. Rupasmita, S. K. Mishra – draft writing.

A. K. Verma, M. Kumar – review & editing.

Conflict of interest:

The authors declare no conflict of interest.

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СИНТЕЗ И ОПРЕДЕЛЕНИЕ ХАРАКТЕРИСТИК НАНОЧАСТИЦ СЕРЕБРА НА ОСНОВЕ ЭКСТРАКТА КОРЫ ТЕРМИНАЛИИ АРДЖУНЫ: ОПТИМИЗАЦИЯ ТЕХНОЛОГИЧЕСКИХ ПАРАМЕТРОВ С ИСПОЛЬЗОВАНИЕМ МЕТОДОЛОГИИ ПОВЕРХНОСТИ ОТКЛИКА И ОЦЕНКОЙ АНТИБАКТЕРИАЛЬНОЙ АКТИВНОСТИ

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Наночастицы (NPs), синтезированные с помощью метода зеленого синтеза, в последнее десятилетие стали рассматриваться как экологически чистая и устойчивая стратегия. В этом процессе экстракт коры терминалии арджуны (*T. arjuna*) действует как восстанавливающий и укупоривающий агент для синтеза наночастиц серебра (AgNPs). Различные биологически активные соединения, такие как сапонины, дубильные вещества и флавоноиды из экстракта коры, играют важную роль в синтезе и стабилизации наночастиц. Появление темно-коричневого цвета в растворе визуально подтверждает образование наночастиц. Кроме того, пик поглощения при длине волны 410 нм подтверждает биосинтез AgNPs. Кроме того, параметры процесса (температура, время перемешивания и концентрация нитрата серебра) для синтеза наночастиц были статистически оптимизированы с помощью методологии поверхности отклика (RSM). Максимальный синтез наночастиц был достигнут при 5 мм нитрата серебра, температуре 80 °C и 40-минутном перемешивании. Сферическая структура AgNPs с диапазоном размеров 10-120 нм и средним размером частиц 36 нм была обнаружена с помощью просвечивающей электронной микроскопии. Кроме того, метод рентгеновской дифракции (XRD) показал кристаллическую природу синтезированных AgNPs. Кроме того, антибактериальный потенциал наночастиц серебра был протестирован против *Escherichia coli* (*E. coli*) и *Staphylococcus aureus* (*S. aureus*), которые показали значительные зоны ингибирования, что свидетельствует о возможности использования AgNPs против штаммов, устойчивых к антибиотикам. Это исследование раскрывает способность экстрактов коры *T. arjuna* к биогенному синтезу AgNPs с высоким антибактериальным потенциалом, которые могут быть использованы в различных биомедицинских целях.

Ключевые слова: зеленый синтез; наночастицы серебра; *Terminalia arjuna*; методология поверхности отклика; антибактериальная активность

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АРДЖУНАТЕРМИНАЛЫНЫҢҚАБЫҚСЫҒЫНДЫСЫНАНЕГІЗДЕЛГЕНКҮМІСНАНОБӨЛШЕКТЕРІНІҢ СИНТЕЗІ ЖӘНЕ СИПАТТАМАЛАРЫН АНЫҚТАУ: ЖАУАП БЕТІНІҢ ӘДІСТЕМЕСІН ҚОЛДАНА ОТЫРЫП ЖӘНЕ БАКТЕРИЯҒА ҚАРСЫ БЕЛСЕНДІЛІКТІ БАҒАЛАЙ ОТЫРЫП, ТЕХНОЛОГИЯЛЫҚ ПАРАМЕТРЛЕРДІ ОҢТАЙЛАНДЫРУ

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Жасыл синтез әдісімен синтезделген нанобөлшектер (NPs) соңғы онжылдықта экологиялық таза және тұрақты стратегия ретінде қарастырыла бастады. Бұл процесте Арджуна терминалының (*T. arjuna*) қабық сығындысы күміс нанобөлшектерін синтездеу үшін қалпына келтіретін және жабатын агент (AgNPs) қызметін атқарады. Сапониндер, таниндер және қабық сығындысынан алынған флавоноидтар сияқты әртүрлі биологиялық белсенді қосылыстар нанобөлшектерді синтездеуде және тұрақтандыруда маңызды рөл атқарады. Ерітіндідегі қара қоңыр түстің пайда болуы нанобөлшектердің пайда болуын көзбен растайды. Сонымен қатар, 410 нм толқын ұзындығындағы жұтылу шыңы агнрps биосинтезін растайды. Сонымен қатар, нанобөлшектерді синтездеуге арналған процестің параметрлері (температура, араластыру уақыты және күміс нитратының концентрациясы) жауап бетінің әдістемесімен (RSM) статистикалық оңтайландырылды. Нанобөлшектердің максималды синтезіне 5 мм күміс нитраты, 80 °C температурада және 40 минуттық араластыру кезінде қол жеткізілді. 10-120 нм өлшем диапазоны және 36 нм бөлшектердің орташа өлшемі бар агнрps сфералық құрылымы трансмиссиялық электронды микроскопия арқылы анықталды. Сонымен қатар, рентгендік дифракция (XRD) әдісі синтезделген агнрps-тің кристалдық сипатын көрсетті. Сонымен қатар, күміс нанобөлшектерінің Бактерияға қарсы потенциалы *Escherichia coli* (*E. coli*) және *Staphylococcus aureus* (*S. aureus*) сыналды, олар айтарлықтай тежелу аймақтарын көрсетті, бұл антибиотиктерге төзімді штаммдарға қарсы AgNPs қолдану мүмкіндігін көрсетеді. Бұл зерттеу *T. arjuna* кортекс сығындыларының әртүрлі биомедициналық мақсаттарда қолдануға болатын жоғары Бактерияға қарсы потенциалы бар агнрps биогендік синтездеу қабілетін көрсетеді.

Кілт сөздер: жасыл синтез; күміс нанобөлшектері; *Terminalia arjuna*; жауап бетінің әдістемесі; бактерияға қарсы әрекет