Green synthesis and antimicrobial evaluation of silver nanoparticles mediated by leaf extract of *Syzygium cumini* against poultry pathogens

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The present study was carried out to determine the effect of plant extract of *Syzygium cumini* and their silver nanoparticles (AgNPs) against the poultry pathogens. The plant extract was prepared in methanol, ethanol, ethyl acetate, chloroform, and *n*-hexane. The results of phytochemical screening showed that the leaf extract of *S. cumini* have medicinally important phytochemical constituents. In the present study, AgNPs were synthesised by using an extract of *S. cumini*. Synthesised AgNPs were confirmed by Fourier-transform infrared spectroscopy, ultraviolet–visible, and scanning electron microscopy. A surface plasmon resonance peak in absorption spectra showed absorption at 419 nm. The synthesised nanoparticles were found spherical in shape with the diameter range of 30–120 nm. The antimicrobial activity of crude extract and AgNPs was checked against the poultry bacterial pathogens by the agar well diffusion method. The authors' findings revealed that the crude extract and AgNPs have promising activities against all the test pathogens. It is concluded that the bioactive compounds from the extract of *S. cumini* and extract-mediated AgNPs can be significantly used as an alternate poultry medicine to replace the available antibiotics, which have adverse side effects.

1. Introduction: Poultry sector is one of the fastest-growing segments of agriculture in Pakistan. Production of broilers and eggs has been increasing per year, due to its increasing demand for human consumption. Nowadays various diseases in poultry sector increased mortality rate, cause little productivity, and related contamination of poultry yields for human consumption. There is an increasing interest to discover alternatives to antibiotics against poultry diseases. Catalytic reduction of organic dyes using plant extracts and phytomedicines are the new trends in drug discovery from natural herbs for the treatment of various diseases [1–3]. Poultry undergoes various diseases, such as diarrhoea, respiratory problems, paralysis, salmonellosis, necrotic enteritis, colibacillosis, ulcerative enteritis, mycobacteriosis, spirochetosis, erysipelas, and fowl cholera [4]. Different infectious organisms are involved in the spreading of serious diseases in poultry. The organisms involved in poultry diseases were divided into four groups, i.e. bacterial, fungal, parasitic, and viral infections. Among these classes, bacterial and fungal infections are very common [5].

Syzygium cumini is an evergreen medicinal plant of family *Myrtaceae*, generally known as jamun in Urdu. *S. cumini* is an evergreen large medicinal plant and distributed throughout Pakistan. Different parts including fruit and bark possess therapeutic properties [6]. Plant extract contains active biomolecules such as polyphenols, which have the ability to reduce silver ions for the synthesis of silver nanoparticles (AgNPs). Silver has a remarkable importance due to its strong antibacterial properties [7]. Various techniques used for the synthesis of AgNPs include chemical, photochemical, biological, γ-radiation, laser, and electrochemical methods but the chemical reduction method is the most common and popular method used for the preparation of AgNPs [8, 9]. AgNPs have a key role in microbiology due to their strong antimicrobial property [10–13]. AgNPs have various biological applications in drug targeting, bioimaging, wound dressing, transfection vectors, labelling agents, and strong antibacterial activity [11–13].

Phytomedicines [13–15] and medicines based on metallic nanoparticles of arthropods metabolites are mostly used in developing countries [16]. Different bioactive constituents are present in these traditional medicines. These medicines are a very effective remedy against different diseases. An extra advantage of these phytomedicines is that their availability at affordable cost, fewer side effects, and easily accessible [14, 16, 17].

There is an increasing interest to discover alternatives to antibiotics against different diseases. Traditional drugs are the new trends in drug discovery from natural herbs against the treatment of various infections [18]. Keeping in view all the medicinal significance of *S. cumini,* collected from district Peshawar, the present study was designed to explore the less investigated side of plant extract and synthesis of their respective AgNPs to examine their antimicrobial potential against poultry pathogens.

2. Materials and methods

2.1. Collection and botanical identification of plant: Fresh leaves of *S. cumini* were collected from different regions of district Peshawar in June 2016. Taxonomic identity of the plant was confirmed in the Department of Botany, the University of Peshawar, and a voucher of the plant was submitted for future reference.

2.2. Drying and grinding: The leaves of *S. cumini* were first thoroughly washed with distilled water and soaked in detergent to remove the dust and microbial load on the surface of leaves.

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Collected leaves were shade dried in dark at room temperature for at least 2–3 weeks. After shade drying the plant leaves were converted into powder form [19].

2.3. Extraction: After the process of drying and grinding, extraction was made. The fine powder of leaves of *S. cumini* was soaked in different organic solvents, i.e. methanol (MeOH), ethanol (EtOH), ethyl acetate (EtOAc), chloroform ($CHCl_3$), and *n*-hexane for 7 days. 100 g of plant powder were soaked in a flask containing 1 l of MeOH, EtOH, EtOAc, $CHCl_3$, and *n*-hexane was shaken well until the plant powder mixed with the solvents. The method was repeated three times to concentrate the extract then separated by using a rotary evaporator [19].

2.3.1. Determination of extractive values: Five grams of plant powder were extracted with 100 ml of respective solvents (i.e. MeOH, EtOH, EtOAc, $CHCl_3$ and *n*-hexane) in a flask for about 24 h, then continuously shaked, kept overnight, decanted, and filtered. The filtrate of each solvent was dried and the remaining extract was then weighed in percentage [20]

$$\%\,\text{Extract} = \frac{\text{Weight of extract obtained in grams}}{\text{Weight of plant material taken in grams}} \times 100$$

2.3.2. Determination of ash value: Five grams of dried powdered material of *S. cumini* were taken in the crucible and burnt on the burner in the open air. It was then kept at 600°C in the furnace for 3 h after then it was cooled. The ash obtained was weighed and the percentage was then calculated with reference to the air-dried sample of *S. cumini* [20]

$$\%\text{Ash} = \frac{\text{Weight of plant material taken after burning}}{\text{Weight of plant material taken before burning}} \times 100$$

2.3.3. Determination of moisture value: Five grams of dried powder of *S. cumini* were taken in already weighed Petri dish. It was then kept in an oven at 110°C for 3 h. The percentage of moisture present in the plant dried powder was then found and calculated with reference to the air-dried sample of the plant [20]

$$\%\text{Moisture} = \frac{\text{Weight of moisture in plant material taken in grams}}{\text{Weight of plant material before heating in grams}} \times 100$$

2.4. Collection of poultry pathogens: Five different bacterial strains used in the present study were collected from the bacteriology section of the Veterinary Research Institute (V.R.I) Peshawar. *Escherichia coli, Salmonella typhi, Proteus vulgaris, Staphylococcus aureus,* and *Klebsiella pnuemoniae* were used in the present study. Confirmed samples were inoculated with the help of a sterile wire loop in sterile nutrient broth tubes from the bacteriology lab V.R.I Peshawar. All the samples were immediately transported to the Microbiology lab at Abasyn University Peshawar for further processing.

2.5. Fourier-transform infrared spectroscopy (FTIR) study: FTIR of all the fractions including MeOH, EtOH, EtOAc, $CHCl_3$, *n*-hexane, and AgNPs was carried out in the PCSIR laboratories. The FTIR Model IR Pretige-21 (Shimadzu Japan) was used in the present study.

2.6. Phytochemical analysis: All the fractions of leaves of *S. cumini* were evaluated for the presence of different phytochemical constituents including tannins, flavonoids, saponins, steroids, alkaloids, anthraquonine, phenol, carbohydrate, reducing sugar, and amino acids. The present study was aimed at finding the phytochemicals (secondary metabolites) from the leaves of *S. cumini* [6, 14].

2.7. Extract-mediated AgNPs: Silver nitrate (BDH Laboratory, BH.15-1TD) was purchased from the local market (Peshawar). Initially 50 g of finely powdered leaves of *S. cumini* were mixed with 500 ml deionised water and boiled for 5 min. By using fresh deionised water, the mixture was centrifuged for 15 min at 5000 rpm at 40°C. Deionised water was used to adjust the extract volume and filtered by Whatman No. 1 filter paper. Then, 90 ml of aqueous 1 mM $AgNO_3$ solution was mixed with 10 ml of extract. Deionised water was used throughout the reaction. $AgNO_3$ solution was used for the reduction of Ag^+ ions. Then, the solution was incubated under dark conditions at room temperature for 24 h. After the incubation period, the solution was again centrifuged at 10,000 rpm for 25 min, to separate the synthesised AgNPs then washed thrice with deionised water and stored for further use [2, 3, 10]. The green synthesis of AgNPs found the presence of high polar constituents in *S. cumini* leaf extract. The amount of polyphenols actually helps in the determination of the size and distribution of the synthesised AgNPs.

2.8. Characterisation of AgNPs: Synthesised AgNPs were characterised by ultraviolet–visible (UV–Vis) and FTIR which showed the size, diameter, morphology, and shape of nanoparticles. AgNPs synthesised from aqueous extract of plant leaves were evaluated through a UV spectrophotometer in a range of wavelengths from 200 to 800 nm.

2.9. Antibacterial screening: Agar well diffusion technique was used for the determination of antibacterial activity. The leaf extract of *S. cumini* was obtained from different solvents and tested against the bacterial species, isolated from poultry. The antibacterial activity of crude extracts and AgNPs was checked against *E. coli, S. typhi, P. vulgaris, S. aureus,* and *K. pnuemoniae.* Nutrient agar medium was prepared according to the manufacture's guidelines and autoclaved at 121°C for 15 min. After autoclave, the medium was then dispensed into 25 mm sterile agar plates and left for solidification. For the confirmation of sterility, prepared agar plates were incubated at 37°C for 24 h. The Uniform lawn of each test species was prepared by spreading on the entire surface of the plates. 10 mg plant extracts were dissolved in 1 ml of dimethyl sulphoxide (DMSO) followed by subsequent addition of 50, 75, and 100 µl of plant extracts. The same concentrations and method were used for the AgNPs. Gentamicin disc and DMSO were used as positive and negative control. All the plates were incubated at 37°C for 24 h. After the incubation period, the zone of inhibition (ZOI) was measured in millimetres by using a ruler [19, 21].

3. Results and discussion: Different bacterial strains included in the current study were collected from the bacteriology section of the V.R.I Peshawar, i.e. *E. coli, S. typhi, P. vulgaris, S. aureus,* and *K. pneumonia.* The plant extract was prepared in MeOH, EtOH, EtOAc, $CHCl_3$, and *n*-hexane. Antimicrobial activity of *S. cumini* and AgNPs was determined through standard procedures and significant results were observed. Whereas the ash and moisture values were also calculated (Table 1). Ash value is important to determine the purity and qualitative standard of samples. High Ash values indicate the contamination and substitution of extract vice versa [22]. The Ash value indicates that plant is best for drug action. The recorded ash value was 21.29%.

Moisture in the extract can cause oxidation which led the compounds present in the extract to become unstable. In addition, the high water content can cause fungi to grow easily. Therefore the determination of moisture contents is very important. Low-moisture content (7.1253%) was detected, which is in the

Table 1 Determination of extractive, ash, and moisture values

Ash value, %	Moisture value, %	Extractive values, %				
		MeOH	EtOH	EtOAc	$CHCl_3$	*n*-hexane
21.2921	7.1253	16	14	9	6	5

acceptable range of 8–10% to avoid fungal growth [23]. The extractive values recording of the crude extract is an approximate measure of a certain constituent availability in a specific fraction. The MeOH fraction have a high extractive value (16%) followed by EtOH (14%), EtOAc (9%), $CHCl_3$ (5%), and *n*-hexane (5%), respectively.

3.1. FTIR results: FTIR of all the fractions including MeOH, EtOH, EtOAc, $CHCl_3$, and *n*-hexane was carried out in the PCSIR laboratories (Peshawar). Different peaks of active compounds were detected in each fraction of the extract (Figs. 1–5).

3.2. Phytochemical analysis of leaves of *S. cumini*: All the fractions of leaves of *S. cumini* were evaluated for the presence of different phytochemical constituents including tannins, flavonoids, saponins, steroids, alkaloids, anthraquonine, phenol, carbohydrate, reducing sugar, and amino acid. Overall results showed the presence and absence of different compounds in each fraction (Table 2).

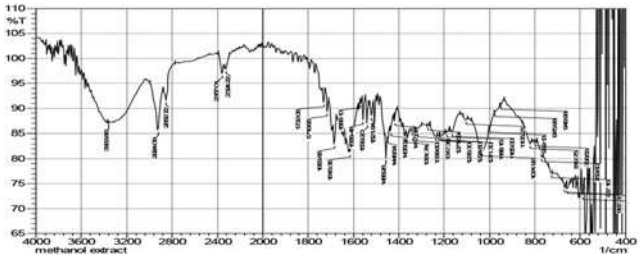

Fig. 1 *FTIR analysis of MeOH fraction of S. cumini leaves*

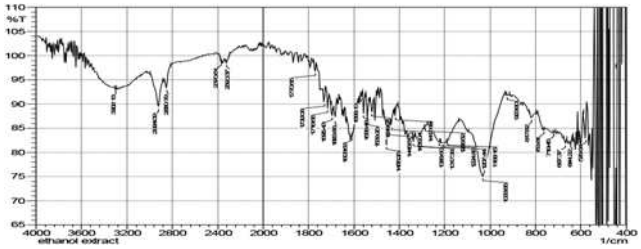

Fig. 2 *FTIR analysis of EtOH fraction of S. cumini leaves*

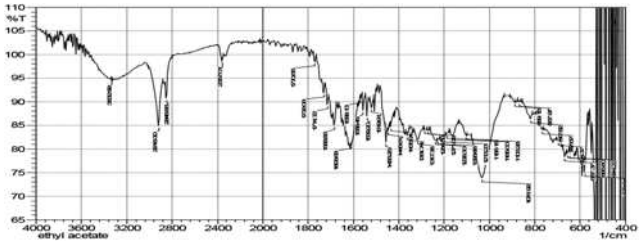

Fig. 3 *FTIR analysis of EtOAc fraction of S. cumini leaves*

3.3. Characterisation of AgNPs and antibacterial activities: Herein, AgNPs were efficiently synthesised by using leaves extract of *S. cumini* and silver nitrate. Silver ions were reduced to AgNPs. AgNPs production was observed by the change in colour of the solution from light yellowish to dark brownish. This colour change took almost 18–20 h at room temperature. The appearance of light yellowish to dark brownish colour is the indicator of the formation of AgNPs in the medium. The intensity of brown colour increased with the incubation time. The pH of the solution was also changed from 6.5 to 5.0, which showed the synthesis of AgNPs (Figs. 6*a* and *b*).

3.4. FTIR of AgNPs: FTIR analysis was carried out to identify AgNPs associated with biomolecules. Different peaks of active compounds were identified corresponding to functional groups of various secondary metabolites (Fig. 7). The O–H peak corresponding to phenol and alcohol was recorded at 3196 cm^{-1} while a peak at 2920 cm^{-1} indicated the presence of C–H alkane bond. The aromatic C=C–C symmetric stretching was observed at 1600 cm^{-1} and C–H bending showed a peak at 1371 cm^{-1}. The respective peaks at 1323 and 1178 cm^{-1} for C–O ether and esters were also recorded. Thus, the formation of AgNPs was confirmed by the changing patterns of peaks of FTIR spectra when compared to other spectra of fractions.

3.5. UV–Vis spectroscopy: The maximum peak absorbance with a high band was observed at 419 nm, a characteristic wavelength showed the existence of AgNPs (Fig. 8).

3.6. Scanning electron microscopy (SEM) analysis of extract mediated AgNPs: The SEM images showed relatively spherical shape AgNPs in the form of clusters with a diameter ranging from 30 to 120 nm. The SEM images showed that the nanoparticles were in single and bunch forms, as shown in Fig. 9. The amount of

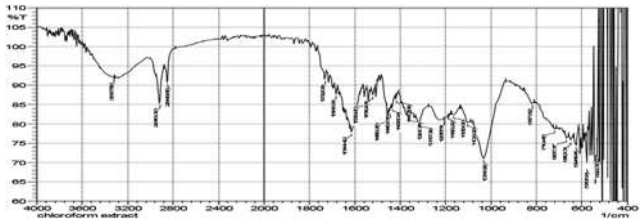

Fig. 4 *FTIR analysis of $CHCl_3$ fraction of S. cumini leaves*

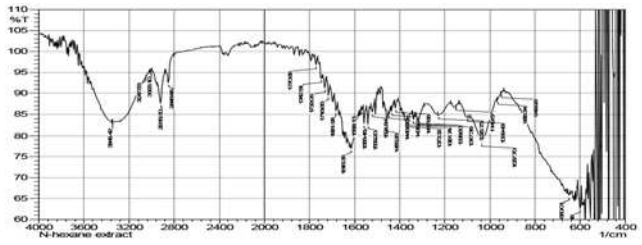

Fig. 5 *FTIR analysis of n-hexane fraction of S. cumini leaves*

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Table 2 Phytochemical analysis of leaves extract of *S. cumini*

S.No	Phytochemicals	MeOH	EtOH	EtOAc	$CHCl_3$	*n*-hexane
1	tannins	+	+	+	+	+
2	flavonoids	+	+	+	+	+
3	saponins	+	+	+	+	+
4	steroids	—	—	—	—	—
5	alkaloids	+	+	+	+	+
6	anthraquonine	+	+	+	+	—
7	phenol	+	+	+	+	—
8	carbohydrate	+	+	+	+	+
9	reducing sugar	+	+	+	+	+
10	amino acid	—	—	—	—	—

+ Sign shows the presence, while — sign shows the absence.

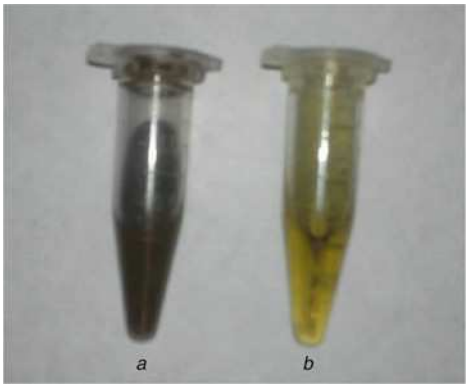

Fig. 6 *Colour indications for the AgNPs*
a Synthesised AgNPs
b Leaf extract of *S. cumini*

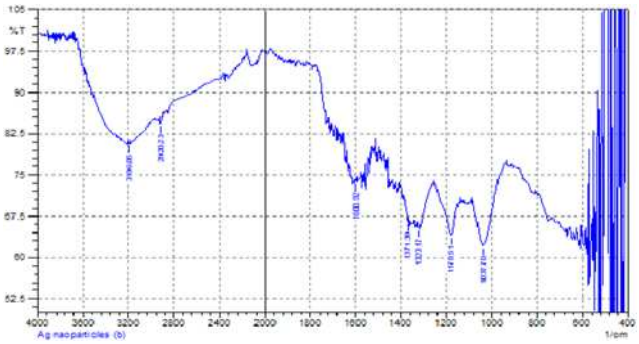

Fig. 7 *FTIR spectra of AgNPs for functional groups*

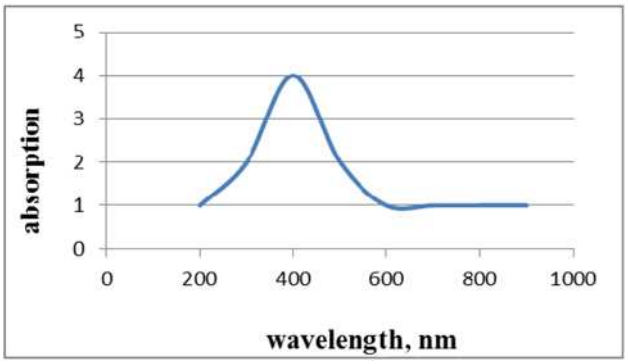

Fig. 8 *UV–Vis spectroscopy*

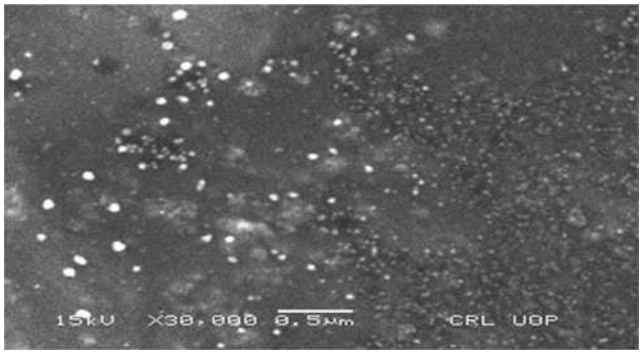

Fig. 9 *SEM analysis of extract-mediated AgNPs*

polyphenols actually helps in the determination of the size and distribution of the synthesised AgNPs.

3.7. Antibacterial activity of *S. cumini* extract: For antibacterial screening, *S. cumini* extracts were obtained from five different organic solvents (i.e. MeOH, EtOH, EtOAc, $CHCl_3$, and *n*-hexane) and were screened against five bacterial strains (*E. coli*, *S. typhi*, *S. aureus*, *P. vulgaris,* and *K. pneumonia*). The triplicate experiments were carried out using the agar well diffusion method and results were reported in ZOI expressed in millimetres (Table 3). Nevertheless, the antibacterial evaluation of MeOH fraction showed significant activities against poultry pathogens in dose-dependent manner as compared to the positive control. All the fractions showed an inhibitory effect at high concentrations. MeOH fraction was more active due to its polarity. Alkaloids, steroids, flavonoids, phenols, saponins, glycosides, terpenoids, tannins, and cardiac glycosides were detected in the MeOH of *S. cumini* [24]. Among poultry pathogens, *E. coli*was found most resistant to all fractions particularly at high concentrations.

3.8. Antibacterial activity of AgNPs: Extract-mediated AgNPs of *S. cumini* were used against poultry pathogenic bacteria including *K. pnuemoniae, S. auerus*, *P. vulgaris, E. coli* and *S. typhi*. AgNPs at 50, 75, and 100 µl and ZOI values are shown in Table 4. Here DMSO expressed no activity while Gentamicin showed (20, 21, 16, 21 and 14 mm) ZOI against *K. pnuemoniae, S. aureus*, *P. vulgaris, E. coli*, and *Salmonella* spp., respectively (Table 4).

The significant results were shown by AgNPs against all pathogens as compared with fractions. The synthesised AgNPs also showed inhibitory potential in a dose-dependent manner. Metal nanoparticles have potential against pathogenic bacteria. The properties of metal nanoparticles can be enhanced through the green synthesis approach. Biological synthesis of nanoparticles attracts

Table 3 Antibacterial activity of leaf extracts of *S. cumini*

S. cumini	Conc., µl	*E. coli* (ZOI)	*S. typhi* (ZOI)	*S. aureus* (ZOI)	*P. vulgaris* (ZOI)	*K. pneumonia* (ZOI)
MeOH	50	10 ± 0.86	9 ± 0.04	15 ± 0.01	17 ± 0.01	15 ± 0.58
	75	18 ± 0.75	16 ± 0.55	18 ± 0.02	18 ± 0.46	15 ± 0.33
	100	21 ± 0.00	27 ± 0.01	21 ± 0.45	21 ± 0.05	18 ± 0.97
	+ve	16 ± 0.23	23 ± 0.58	12 ± 0.28	16 ± 0.32	18 ± 0.03
EtOH	50	13 ± 0.30	15 ± 0.78	15 ± 0.21	11 ± 0.00	10 ± 0.31
	75	19 ± 0.83	23 ± 0.00	17 ± 0.79	14 ± 0.76	17 ± 0.00
	100	24 ± 0.00	20 ± 0.04	19 ± 0.34	18 ± 0.28	18 ± 0.73
	+ve	21 ± 0.78	17 ± 0.31	19 ± 0.01	16 ± 0.02	21 ± 0.03
EtOAc	50	17 ± 0.29	18 ± 0.20	13 ± 0.02	13 ± 0.39	11 ± 0.01
	75	19 ± 0.74	21 ± 0.36	14 ± 0.30	17 ± 0.47	14 ± 0.37
	100	23 ± 0.40	25 ± 0.00	14 ± 0.39	19 ± 0.26	18 ± 0.73
	+ve	19 ± 0.49	14 ± 0.02	19 ± 0.74	18 ± 0.02	15 ± 0.83
$CHCl_3$	50	16 ± 0.38	12 ± 0.73	15 ± 0.79	13 ± 0.89	16 ± 0.73
	75	20 ± 0.85	17 ± 0.00	17 ± 0.38	16 ± 0.36	18 ± 0.21
	100	25 ± 0.77	23 ± 0.99	19 ± 0.70	20 ± 0.72	20 ± 0.37
	+ve	13 ± 0.20	23 ± 0.43	16 ± 0.01	21 ± 0.03	17 ± 0.00
n-C_6H_{14}	50	8 ± 0.76	9 ± 0.00	12 ± 0.34	9 ± 0.66	12 ± 0.02
	75	11 ± 0.26	10 ± 0.35	13 ± 0.37	10 ± 0.47	14 ± 0.76
	100	15 ± 0.38	14 ± 0.40	13 ± 0.02	14 ± 0.00	17 ± 0.85
	+ve	14 ± 0.00	19 ± 0.03	19 ± 0.70	16 ± 0.02	19 ± 0.01

Gentamicin was used as +ve and DMSO was used as −ve control

Table 4 Antibacterial activity of *S. cumini* AgNPs against poultry pathogens by agar well diffusion method

Conc., µl	ZOI, mm						DMSO
	K. pneumonia	*S. auerus*	*P. vulgaris*	*E. coli*	*S. typhi*	−ve	
50	18 ± 0.21		15 ± 0.58	18 ± 0.00	18 ± 0.71	19 ± 0.53	—
75	19 ± 0.73	17 ± 0.71	19 ± 0.73	19 ± 0.20	21 ± 0.17	—	—
100	28 ± 0.43	30 ± 0.04	23 ± 0.38	29 ± 0.30	23 ± 0.53	—	—
+ve	20 ± 0.00	21 ± 0.03	16 ± 1.48	21 ± 0.00	14 ± 0.35	—	—

Table 5 Comparative literature review

S. No	This work	Published work	Refere-nces
1	this work deals with the antimicrobial screening of crude extract and AgNPs synthesised from *S. cumini* leaf extract against poultry pathogens. No study related to their antioxidant properties was carried out.	plant extract-mediated AgNPs were synthesised using two medicinally and economically important plants, *A. bilimbi* and *S. cumini*. Their AgNPs were characterised and evaluated for their free radical scavenging potentials only to have a comparative study.	[27]
2	this study showed both green synthesis and antimicrobial evaluation of leaf extract and their respective AgNPs prepared from *S. cumini* plant. Here AgNPs were synthesised by using different ratio of leaf extract and silver nitrate solution than the reported method.	a comparative study of leaf and seed extracts along with their green synthesised AgNPs from *S. cumini* were carried out. No antimicrobial screening of either the plant extract or their respective AgNPs was performed.	[28]
3	we studied antibacterial potential of both *S. cumini* leaf extract and their green synthesised AgNPs against poultry pathogens.	here antibacterial activity of *S. cumini* leaf extracts against multidrug resistant pathogenic bacteria was studied but they neither synthesised their AgNPs of extract nor were they evaluated for antibacterial potential.	[29]
4	our work deals not only with the antimicrobial activities of *S. cumini* leaf extract but also with their green synthesised AgNPs, respectively	this work did not report about the green synthesis and antimicrobial screening of AgNPs of *S. cumini* leaf extract	[30, 31]

great attention due to its eco-friendly nature and promising results [25].

Poultry is one of the most important food economies all over the world including Pakistan. Approximately 90 billion tonnes of chicken meat is produced every year [26]. A large diverse antibiotic of human use is applied to enhance poultry in Pakistan, as a result, antibiotics resistance is developed in pathogens. This results in failure of treatment, economic loss and could act as a gene pool to transmit to humans. Similarly, the antibiotics present in meat, egg, and animal products also act as a serious threat to human health. The current study focuses on poultry pathogens instead of human pathogens. Furthermore, extract-mediated AgNPs of

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S. cumini were also screened against these pathogens to search a new, alternate, eco-friendly, and economical source to be used in the poultry industry.

A comparative literature review of our research work and already published work is given in Table 5 for better understanding and support of the importance of our reported study.

4. Conclusion: It concluded that the valuable phytochemicals present in *S. Cumini* leaf extract were the reason for stable AgNPs formation in solution with good surface plasmon behaviour. The colour change from light brown to dark brownish accompanied by simultaneous pH change from 6.5 to 5.0 gives the first indication of AgNPs formation. Further characterisation was carried out by FTIR, UV, and SEM analysis. The appearance of AgNPs in clusters and their spherical shape with 30–120 nm diameters were observed through the SEM image. The plant leaf extract as well as their AgNPs showed promising antibacterial potential. Thus, we propose that both *S. Cumini* leaf extract and their biosynthesised AgNPs can be significantly used as alternate poultry medicine to replace the available antibiotics after further insight mechanistic studies.

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