Silver and Gold Nanoparticles Induced Differential Antimicrobial Potential in Calli Cultures of *Prunella Vulgaris*

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Abstract

Background: *Prunella vulgaris* is a medicinally important plant containing high-valued chemical metabolites like Prunellin which belong to family *Lamiaceae* and it is also known as self-heal. In this research, calli culture exposed to differential ratios of gold (Au) and silver (Ag) nanoparticles (1:2, 1:3, 2:1 and 3:1) along with naphthalene acetic acid (2.0 mg NAA) were investigated for antimicrobial potential. A well diffusion method was used for antimicrobial properties.

Results: Here, two concentrations (1 and 2 mg/6 µl) of all treated calli cultures and wild plants were used against *E. coli*, *P. aeruginosa*, *S. typhi*, *B. atrophaceus*, *B. subtilis*, *A. tumefaciens*, *E. caratovora* and *C. albicans*. DMSO and antibiotics were used as negative and positive controls. Here, the calli exposed to gold (Au) NPs + 2.0 mg NAA displayed the highest activity (25.7 mm) against *S. typhi* than other extracts, which was considered the most susceptible species, while *A. tumefaciens* and *C. albicans* was the most resistance species. A possible mechanism of calli induced nanoparticles was also investigated for cytoplasmic leakage.

Conclusion: From the above data it is concluded that the medicinal *Prunella vulgaris* is an important plant for the development of anti-microbial drugs and applicable in various pharmaceutical research.

Introduction

Nanotechnology is a versatile field and their purpose is increasing rapidly in almost all discipline of science. Nanotechnology promotes speedily and estimated that it will revolve toward a trillion-dollar industry at 2021. It has a great influence on industrial revolution [1]. Because of the particular feature of this subject is promoting its approach in different discipline of science. Contrarily, the application of nanotechnology is new in the field of research and it need to progress in plant tissue culture and medicinal plant science. Presently, most of the previous reports with reference to plant growth, seed germination and physiological parameters are anxious regarding to nanoparticles applications [1, 2]. Out of all metal nanoparticles, mainly the gold and silver getting more attention because of their physico-chemical applications [3]. Through silver and gold nanoparticles the growth of microorganisms can be prevented, but this effect is based on the shape and size of nanoparticles [4]. Through gold and silver nanoparticles the genes can be transferred to the target cell in in-vitro [5]. The silver and gold nanoparticles are utilized for the identification of protein. The gold nanoparticles are also being useful in the recognition of antigen that is fused to the antibody [6].

Microbes are evolving to become resistances to particular antibiotics through mutation process [7] and the resistance ability is widespread in microbial population by the process of horizontal gene transfer [8]. Furthermore, as microorganisms become resistant to particular antibiotics, the subsequent use of alternative antibiotic might select for additional resistance, which give rise to the multi-drug resistance strains [9, 10, 11].

The family *Lamiaceae* contains perennial plant called *Prunella vulgaris*. This is a herbaceous plant and used for eating in many parts of the world. Due to their wound-healing properties, it is also called self-heal or all heal [12]. *Prunella vulgaris* is grown in different areas of Pakistan including KPK, Kashmir, Swat and Murree at the height of about 1800 to 3300 meters [13]. Still this plant is used in conventional medicine in case of cold, headache, sore throat and nephritis in many countries including China, Korea and Greece [14]. *Prunella vulgaris* is a significant medicinal plant because it having natural phytochemicals. In southeast China the dried plant is used for drinking [15].

Prunella vulgaris is effective against pain and to minimize the high fever and high blood pressure of the body [16, 17]. Currently, many studies show that this medicinal plant has a wide domain of medicinal pursuit counting anti-cancerous, anti-septic, anti-spasmodic, anti-HSV and anti-rheumatic properties [18, 19]. *Prunella vulgaris* is also

effective in case of HIV and herpes viruses and it is functional to treat lymphatic system, and tuberculosis [20]. The best result of *Prunella vulgaris* against bacteria and viruses has been reported. It has been investigated for antimicrobial activities against *E. coli*, *S. aureus*, and *P. aeruginosa*. This plant has the ability to inhabit many gram-negative bacteria in in-vitro condition. Prunellin a polysaccharide compound present in this medicinal herb work as repressing against HIV virus [21]. This medicinal herb displayed good effects as anti-mutagenic. The spike of *Prunella vulgaris* taken as a sample and after tasting it shows a good result in opposition to carcinogens and environmental mutagens [22].

Many substances such as flavonoid, coumarins, organic acid, sterols and triterpenes have been extracted from *Prunella*. The aqueous solution of *Prunella vulgaris* containing Prunellin was then purified and categorized which displayed optimal anti-HIV activity [23, 24]. The vulgarisin A, a substance found in this medicinal plant has the ability to inhibit the cancer cell line in human body. Triterpenes substance composed of ursolic acid, oleanolic acid and botulinic acid is also extracted from this plant. Oleanolic acid out of them is effective as anti-inflammatory, anti-fungal as well as helpful in curing lung cancer [25, 26]. *Prunella vulgaris* contain Rosmarinic acid is a polyphenol act as anti-tumor. In *Prunella vulgaris* the bioavailable components characterized which contain tannins (52.25 mg), proteins (441.6), phenolics (55.78 mg), saponins (350 mg) carbohydrates (375 mg; [27]. But the importance substances of *Prunella vulgaris* are Prunellin and rosmarinic acid [24]. The healing property of this medicinal plant is due to the presence of phenolic acid, while the anti-inflammatory activity is given by the ursolic and oleanolic acid [28]. *Prunella vulgaris* help to block the nerves system inflammation and liver disorder due to the presence of caffeic acid [29]. Therefore, the overall objective of the current study was to investigate the wild plant as well as the greener synthesize nanoparticles prepared from the NAA-induced calli cultures for antimicrobial potential for possible novel drug design against resistant bacterial species.

Materials And Methods

Explant selection and proliferation

The *Prunella vulgaris* healthy and fresh plants were collected from natural habitat of District Swat, KPK, Pakistan. To develop callus cultures, green and undamaged leaves of *Prunella vulgaris* were excised as optimal explants. The plant leaf was cut into pieces of about 3–4 mm². The explants were sterilized as stated by the procedure of Ahmad *et al.* [30]. For callus culture, MS media with 2.0 mg/l NAA was used [31]. Then 2.3-gram of MS media and 15-gram sucrose was added to flask containing distilled water. For solidification of media, 4.5-gram agar was added. The flasks containing culture media were sterilized in an autoclave at 121 °C, 15 psi for 20 minutes. The leaf pieces were incubated onto MS media for 30 days to develop callus. To examine the effect of differential ratios of nanoparticles (NPs) and naphthalene acetic acid (NAA) on callus propagation, fresh callus was taken at the end of 1 month of explants incubation on NAA augmented media. The calli cultures were then shifted to the different fresh media which composed of differential ratios of gold (Au) and silver (Ag) nanoparticles alone or in mixture with NAA [32]. The media are expressed as a treatment in Table 1.

Table 1

NPs and NAA applied for callus culture of *prunella vulgaris*. To examine the effect of diverse nanoparticles (NPs) and naphthalene acetic acid (NAA) on callus propagation, fresh calli cultures were taken at the end of 1 month of explants incubation on NAA media. The calli cultures were then sub-culture to fresh media which composed of differential ratios of gold (Au) and silver (Ag) nanoparticles alone or in mixture with NAA.

Treatment	NPs + NAA (Auxin)
T1	Full MS with 2.0 NAA
T2	Wild plant
T3	½ MS with 2.0 NAA
T4	AgAu 1:2 + 2.0 NAA
T5	AgAu 1:3 + 2.0 NAA
T6	AgAu 2:1 + 2.0 NAA
T7	AgAu 3:1 + 2.0 NAA
T8	Au + 2.0 NAA
T9	Ag + 2.0 NAA

Calli cultures preparation for antimicrobial activities

In this study, to investigate the antimicrobial activities, the calli cultures were taken out from MS media, gently washed the calli with clean distilled water, filter paper was used to eliminate the excess water (Whatman Ltd, England) and then the calli cultures were weighed as a fresh weight (FW). To determine the dry weight (DW), an oven was used to dry the calli (Thermo Scientific; Germany) at 50 °C up to 24 hours and the calli were weighed. The dry weight (DW) and fresh weight (FW) of the calli were stated in gram/100 ml (Table 2).

Table 2

Fresh and dry weight of *Prunella vulgaris* extract. To know the fresh weight (FW) the inoculated calli were taken and then weighed. For extract preparation, wild plants extract and the fresh calli were dried in an oven and then weighed (DW).

Media used	Callus fresh weight (g)	Callus dry weight (g)
Full MS 2.0 NAA	1.1606	0.292
Wild plant	0.4972	0.0559
½ MS 2.0 NAA	1.522	0.0321
AgAu 1:2 + 2.0 NAA	0.1770	0.0163
AgAu 1:3 + 2.0 NAA	0.1275	0.0214
AgAu 2:1 + 2.0 NAA	0.5323	0.0482
AgAu 3:1 + 2.0 NAA	0.1905	0.0119
Au + 2.0 NAA	0.1337	0.011
Ag + 2.0NAA	0.598	0.039

Fazal *et al.* [33, 34], protocol was used for the preparation of extract. The callus of *Prunella vulgaris*, were dried in an oven at 50 °C to obtain the dried powder, from which the extract was prepared for anti-microbial activities. Here, 5 g of dried power were taken in sterilized flask. Then 50 ml of ethanol were poured to each flask and incubated for 7 days with period shaking and finally filtered. The filtrated extract was collected after 1 week incubation. The excess substance was removed from extract by using rotary evaporator at 40 °C. This experiment was 3 times repeated and every time fresh volume of ethanol was used. The Whatman filter paper No 1 was utilized for the filtration of final crude extract isolation. To concentrate the final extract (ethanol) the rotary evaporator at 40 °C was used, individually for each extract. After evaporation the extract was kept at 4 °C in a flask with tightly packed.

Microorganisms tested for antimicrobial potential

The microorganisms which were used, namely, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*), *Erwinia caratovora* (*E. caratovora*) and *Agrobacterium tumefaciens* (*A. tumefaciens*; Gram-negative bacteria) *Bacillus atrophaeus* (*B. atrophaeus*), *Bacillus subtilis* (*B. subtilis*), (Gram-positive bacteria). The above bacteria were collected from Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar, Pakistan. One fungal strain, *Candida albicans* (Gram-positive fungus) was used and obtained from the same place (PCSIR, Peshawar, Pakistan). To maintained, microorganisms were placed on solid media at 4 °C till activity (Table 3).

Table 3

The microorganisms which were used, namely, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*), *Erwinia caratovora* (*E. caratovora*) and *Agrobacterium tumefaciens* (*A. tumefaciens*; Gram-negative bacteria) *Bacillus atrophaeus* (*B. atrophaeus*), *Bacillus subtilis* (*B. subtilis*), (Gram-positive bacteria). One fungal strain, *Candida albicans* (Gram-positive fungus). The above microorganisms were collected from Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar, Pakistan.

Microorganisms	Type	Source
<i>Escherichia coli</i>	Gram-negative	PCSIR Lab (Peshawar)
<i>Pseudomonas aeruginosa</i>	Gram-negative	PCSIR Lab (Peshawar)
<i>Salmonella typhi</i>	Gram-negative	PCSIR Lab (Peshawar)
<i>Bacillus atrophaeus</i>	Gram-positive	PCSIR Lab (Peshawar)
<i>Bacillus subtilis</i>	Gram-positive	PCSIR Lab (Peshawar)
<i>Agrobacterium tumefaciens</i>	Gram-negative	PCSIR Lab (Peshawar)
<i>Erwinia caratovora</i>	Gram-negative	PCSIR Lab (Peshawar)
<i>Candida albicans</i>	Fungi	PCDIR Lab (Peshawar)

Determination of anti-microbial potential of NPs induced calli cultures

The anti-microbial ability of callus of *Prunella vulgaris* extract was determined through Well-diffuse method [33, 34, 35]. In this method the microbe's suspension were added to nutrient agar media and level off the microbial suspension through sterile swab moistened. In each agar media plate 4 well of about 4–5 mm in diameter were boarded through a sterilized cork borer. Then 24 µl of dimethyl sulfoxide (DMSO) was added to one well of each inoculated media plate as negative control. In second well 24 µl of antibiotics (the antibiotics which was effective against the microbe), were poured to each media plates as positive control. In the third well of all agar media plates were filled with 1 mg of each solvent extract and then to the fourth well 2 mg of each solvent extract to all agar media plates were added. After this procedure the inoculated agar media plates were kept at 32–37 °C for 24 hours. The dimethyl sulfoxide (DMSO) did not show any activity while, antibiotics and solvent extract inhibit the growth of microorganisms, which were present in media. Zones were formed around those wells where the growth of microorganisms was inhibited. These zones were measured in millimeter.

Statistical analysis

The mean zone of inhibition was obtained from triplicated data using Statistix software (V 8.1; USA). The standard deviation of the mean did not exceed 5%. The LSD values was also obtained using Statistix software (V 8.1; USA).

Results

In the current research, the callus cultures of medicinally important *Prunella vulgaris* were grown in PCSIR Peshawar. The wild plants were collected from wild habitat and washed with distilled water to remove the dust particles. In PCSIR Labs Complex, Peshawar-Pakistan, the explants (leaves) were sterilized with 70% ethanol and 0.5-2% NaOCl. The callus was cultured on Murashige and Skoog media containing 2.0 mg/l of naphthalene acetic acid (NAA). To study the out-come of nanoparticles (gold and silver NPs) and naphthalene acetic acid (NAA), the fresh callus was then sub-

culture to agar media containing gold and silver NPs and NAA. In this study, 9 different treatments were tested with NAA either NPs alone or in differential ratios to determine the efficiency of callus proliferation. It was obtained that the calli responded to all treatments containing NPs and NAA. To know the fresh weight (FW), the NPs treated calli cultures were taken and then weighed. For extract preparation, wild plants extract and the fresh NPs treated calli were dried in an oven and then weighed (DW). The fresh and dry weight was shown in Table 2. The extract was prepared for antimicrobial activities and the samples were used against gram-negative bacteria (*E. coli*, *P. aeruginosa*, *S. typhi*, *A. tumefaciens* and *E. caratovora*, gram-positive bacteria (*B. atrophaeus* and *B. subtilis* and a fungi *C. albicans*). Dimethyl sulfoxide (DMSO) and antibiotics were also applied to the microbes for negative and positive controls.

Nanoparticles induced differential antimicrobial potential in Calli cultures of *Prunella*

In this study two different concentrations (1 mg/6 ul and 2 mg/6 ul) of wild plant extracts, NPs-treated calli cultures and callus grown on half and full MS media augmented with 2 mg/l NAA alone was compared for antimicrobial potential against human and plant pathogens. The 1 mg/6 ul extract of full MS media with 2 mg/l NAA was only effective against *S. typhi* and displayed 10 mm zone of inhibition. The 2 mg/6 ul extract was more effective against *B. subtilis* and formed 15.25 mm zone of inhibition while, against *E. caratovora* it displayed 10.65 mm zone respectively. The 2 mg/6 ul extract of wild plant have shown maximum activities against *P. aeruginosa* and *A. tumefaciens* (20.4 mm zone of inhibitions). Furthermore, the 1 and 2 mg/6 ul extract obtained from calli induced by Half MS media with 2.0 mg/l NAA were useful and inhibited the growth of *S. typhi* (10.4 mm and 10.55 mm), *B. atrophaeus* (10 mm and 9.95 mm) and *B. subtilis* (9.95 mm and 15.35 mm). Interestingly, the 1 mg/6 ul extract of Half MS media was more active against plant pathogens including *A. tumefaciens* (20.15 mm) and *E. caratovora* (20.5 mm) as shown in Table 4.

Table 4

Antimicrobial potential of calli cultures of *Prunella vulgaris* induced by NAA and nanoparticles. (Abbreviation of microbes: (E.c) *E. coli*, (P.a) *P. aeruginosa*, (S. t) *S. typhi*, (B.a) *B. atrophaeus*, (B.s) *B. subtilis*, (A.t) *A. tumefaciens*, (E.c) *E. caratovora* and (C.a) *C. albicans*).

Media used	Sample weight	Extract weight	mg/ul	E.c	P.a	S. t	B.a	B.s	A.t	E.c	C.a
Full MS 2.0 NAA	1.1606	0.292	1 mg	–	–	10	–	–	–	–	–
			2 mg	–	10.4	10.3	–	15.25	–	10.65	–
Wild plants	0.4972	0.0559	1 mg	10.5	20	10.6	–	10.5	–	–	10.9
			2 mg	105	20.4	15.3	10.2	15	20.4	10	–
½ MS 2.0 NAA	1.522	0.0321	1 mg	–	10.75	10.4	10	9.95	20.15	20.5	–
			2 mg	–	–	10.55	9.95	15.35	–	–	–
AgAu 1:2+2.0 NAA	0.1770	0.0163	1 mg	–	–	10.15	–	–	–	–	–
			2 mg	10.25	–	10.1	–	10.5	–	–	–
AgAu 1:3+2.0 NAA	0.1275	0.0214	1 mg	9.9	–	–	–	–	–	10.25	–
			2 mg	10.2	–	–	15.25	10.25	–	9.9	–
AgAu 2:1+2.0 NAA	0.5323	0.0482	1 mg	–	10.8	10.7	10.1	15.1	–	10.5	–
			2 mg	–	–	15.25	15.4	20.55	–	15.25	–
AgAu 3:1+2.0 NAA	0.1905	0.0119	1 mg	–	–	10.25	10.2	–	–	15.1	–
			2 mg	–	–	10.35	15.3	–	–	–	–
Au+2.0 NAA	0.1337	0.011	1 mg	9.9	–	10.9	–	–	–	–	–
			2 mg	10.25	–	25.7	–	–	–	–	–
Ag+2.0 NAA	0.598	0.039	1 mg	10	–	10.3	20	10.25	–	–	10.85
			2 mg	10.3	–	10.45	25	10.5	–	–	–
DMSO	–	–	–	–	–	–	–	–	–	–	–
Antibiotics											

Both the extract concentrations of calli obtained from NPs (AgAu 2:1) and NAA (2.0 mg/l) augmented media did not show antimicrobial activities against *E. coli* and *A. tumefaciens*, as well as against fungi (*C. albicans*). The same extracts exhibited optimal activities of 10.7 mm and 15.25 mm against *S. typhi*, while the extracts displayed 10.1 mm and 15.4 mm against *B. atrophaeus*. However, the extracts displayed 15.1 mm and 20.55 mm, and 10.5 mm and 15.25 mm zones of inhibition against *B. subtilis* and *E. caratovora* respectively. The antimicrobial activity of the calli extract grown on higher ratio of NPs (AgAu 3:1) and NAA (2.0 mg/l) were applied against pathogenic microorganisms. Both extracts showed antimicrobial activities and inhibited the growth of *S. typhi* (10.25 mm and 10.35 mm) and *B. atrophaeus* (10.2 mm and 15.3 mm), while *E. caratovora* (15.1 mm) was only inhibit by the 1 mg/6 ul (Table 4). The

silver nanoparticles alone in combination with NAA was more effective against *B. atrophaeus* and displayed 20 mm and 25 mm zones of inhibition. Further, 10.25 mm and 10.5 mm inhibition zones were observed against *B. subtilis*. Here, 1 mg/6 μ l extract showed 10.85 mm activity against the fungal strain *C. albicans*. The calli extract which were taken from gold NPs and NAA (1 and 2 mg/6 μ l) displayed inhibitory potential against *E. coli* (9.9 mm and 10.25 mm) and *S. typhi* (10.9 mm and 25.7 mm). The 2 mg/6 μ l extract exhibited the highest antimicrobial potential of 25.7 mm *S. typhi* in this research. The antimicrobial activities of both extract (1 and 2 mg/6 μ l) was less effective against the bacterial strains, *P. aeruginosa*, *B. atrophaeus*, *B. subtilis*, *A. tumefaciens* and *E. caratovora* as well as to the fungal strain *C. albicans* (Table 4). The calli cultures exposed to AgAu 1:2 + NAA displayed minimum activities against pathogenic bacteria. This extract did not show any potentiation to the other microbes used in this study. The activity of 2 mg/6 μ l extract of calli was also less effective, but maximum than the 1 mg/6 μ l. The highest inhibition rate of this extract was observed against *E. coli* (10.25 mm), *S. typhi* (10.1 mm) and *B. subtilis* (10.5 mm) as shown in Table 4. The antimicrobial activity of 1 mg/6 μ l NPs (AgAu 1:3 + NAA) extracted calli were investigated against multiple microbes and displayed activities against *E. coli* (9.9 mm) and *E. caratovora* (10.25 mm), while no such results was found against the remaining strains of bacteria and fungi. The activity of 2 mg/6 μ l was found to be effective than 1 mg/ μ l. Its highest effect was recorded against *B. atrophaeus* (15.25 mm), *B. subtilis* (10.25 mm), *E. coli* (10.2 mm) and *E. caratovora* (9.9 mm). The extract of this cultures did not show any effect against *P. aeruginosa*, *S. typhi*, *A. tumefaciens* and to the fungal strain *C. albicans*.

Discussion

Microorganisms are the active sources of causing different types of disease in human, plants and animals. Earlier effective antibiotics were used against microorganisms but those antibiotics are not effective in the current era, because antibiotics resistance mechanism were developed by these pathogenic microorganisms. The resistance mechanism in microbes is developing by mutation or genetic recombination [36]. In this study we focus on medicinal plant *Prunella vulgaris* extract alone or the calli grown on MS media augmented with different ratios of NPs and NAA which have displayed strong antimicrobial activities. The medicinal plant extract is less dangerous to the health of humans and animals [37]. The explant derived from leaf of *Prunella vulgaris* plant and its subsequent proliferation to callus was exploited as antimicrobial agent. The calli were than exposed to different concentration of gold (Au) and silver (Ag) nanoparticles alone and with the combination of 2.0 mg NAA (auxin). Yet it is not understandable that how these gold and silver NPs alone or along with NAA control callus growth in medicinal plant including *P. vulgaris* [38]. The present research aimed to determine the antimicrobial ability of various extract obtained from *P. vulgaris* against seven pathogenic bacterial strains and one fungal strain. The antimicrobial activities of *P. vulgaris* plant and calli extracts obtained from different media used in this research was determined by measuring the inhibition zones of two concentrations (1 and 2 mg/6 μ l).

In the current study, the most significant activity of the extract obtained from wild plant which was effective against all of the bacterial species and fungi; however, Rasool *et al.* [39], also proposed that the wild plant is more effective against pathogenic microbe, while the combination of gold (Au) and 2.0 mg NAA was only effective against *E. coli* and *S. typhi*, as Krutyakov *et al.* [40] proposed that silver NPs have better antimicrobial activity than gold. The AgAu 1:3 with 2.0 mg NAA combination was less effective against *S. typhi*, while the other extract showed activities against *S. typha*, as Yallappa *et al.* [41] reported that Ag, Au and Ag-Au NPs showed antimicrobial activities against *S. typhi*. The growth of *C. albicans* was only inhibited by 1 mg/6 μ l extract of wild plant, 10.9 mm of zone was appeared and the 1 mg/6 μ l extract of Ag + 2.0 mg NAA displayed 10.85 mm zone of inhibition, while *C. albicans* show resistance to the other extracts, as Yallappa *et al.* [41] suggest that Ag NPs exhibited optimum antimicrobial activities than Au NPs on fungal strains. The combination of Au + 2.0 mg NAA effect the growth of *S. typhi* and exhibited the highest

inhibition zone of 25.7 mm. Tabrizi *et al.* [42] reported that the antimicrobial activities of mutual Ag-Au are more effective than Ag and Au alone. From the above statement it is observed that the microbial strains *S. typhi* and *B. subtilis* are more susceptible to the *Prunella vulgaris* extract, while Yallappa *et al.* [41] and Bankura *et al.* [43] reported that *S. typhi* and *B. subtilis* are more susceptible to Ag-Au NPs. The bacterial strain *A. tumefaciens* and fungal strain *C. albicans* are more resistance to the different extract of calli grown on multiple media containing combinations of NPs and NAA. Nanoparticles utilized their antimicrobial activities through several mechanisms, including: (1) direct interaction with microbial cell wall; (2) prevention of Biofilm generation; (3) initiation of reactive oxygen species (ROS); and (4) interaction with DNA or proteins [44]. The gold and silver NPs interact with MDR microbes and activate oxidative stress mechanisms. It also inhibits enzymes and proteins as well as epigenetic changes in DNA [45]. The Ag NPs interact with cell membrane and increase their permeability. Ag NPs activate ATP generation and DNA replication in microbes [46]. The Au NPs interact with bacterial cell membrane and lead to cell lysis [47]. To protect from antibiotics the bacteria form Biofilm, in which the bacterial cells are attaching to each other on the surface within extracellular polymeric penetration (EPS) produce by the bacterial cell. EPS help the bacterial cell to become resistances to multiple antibiotics [48]. Most of the studies suggest that NPs prevent Biofilm formation by penetrating the EPS matrix and cause death of the microorganisms [49] as shown in Fig. 1.

Conclusion

Medicinal plant *Prunella vulgaris* is broadly distributed in many countries of the World. This medicinal plant has ironic properties with lower-cost and well performing than synthetic drugs. In this study we notice that the extract of *Prunella vulgaris* alone or calli cultures having a strong antimicrobial activity against different bacteria and fungi. It shows that the compound present in this medicinal plant inhibits the microbial growth. So, the *Prunella vulgaris* can be very useful in making new anti-microbial drugs, and anti-cancerous drugs. Due to its anti-microbial, anti-cancerous and healing properties it gets more attention in different field of biological research.

Abbreviations

FW Fresh weight

DW Dry Weight

Au Gold

Ag Silver

NPs Nanoparticles

NAA Naphthalene acetic acid

mm millimeter

PCSIR Pakistan Council of Scientific and Industrial Research

MDR Multi drug resistance

LSD Least significant differences

MS-media Murashige and Skoog media

DMSO Dimethyl sulfoxide

A. tumefaciens *Agrobacterium tumefaciens*

B. atrophaeus *Bacillus atrophaeus*

B. subtilis *Bacillus subtilis*

C. albicans *Candida albicans*

E. coli *Escherichia coli*

E. caratovora *Erwinia caratovora*

P. aeruginosa *Pseudomonas aeruginosa*

S. aureus *Staphylococcus aureus*

Declarations

Availability of data and materials

Data related to this manuscript will be available on request to the corresponding authors

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not applicable

Competing of interest

All authors declare that they have no potential conflict of interest.

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Author's contribution

NA, JM, KK and WA have optimized callogenesis protocol; HF provide Lab facilities for antimicrobial potential and wrote the whole manuscript. MA, LR and HK prepared nanoparticles, BHA designs the whole experiment and CH help in data interpretation and reviewed the final manuscript for submission to Journal.

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Figures

Figure 1

Different mechanisms of action of NPs in bacterial cell. The gold and silver NPs interact with MDR microbes and activate oxidative stress mechanisms. It also inhibits enzymes and proteins as well as epigenetic changes in DNA.