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Bioactive properties, biosynthesis of silver nanoparticles of *Wedelia chinensis* and its applications

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Abstract
The present investigation involves a study of bioactive substances from plant extracts of *Wedelia chinensis*. Plant phytochemicals contribute to great antimicrobial potential against bacterial cultures. Phytochemical analysis elucidated that *Wedelia chinensis* consist of a broad range of secondary metabolites including alkaloids, saponins, phenols, flavonoids, tannins, carbohydrates, terpenoids, glycosides, quinones, anti-oxidants. The antimicrobial activity of methanol extracts of *Wedelia chinensis* whole plant was studied by well diffusion method against ten bacterial cultures. The synergistic potential of leaf, stem and root extract was also studied and silver nanoparticles (NPs) were synthesized from the leaf, stem and root extracts. Cytotoxicity of the plant extracts and silver NPs was determined by brine shrimp lethality assay. The seed germination of silver NPs with Triticum seeds (wheat) was observed and found effective in seed germination. The obtained outcomes of the present study indicates that the extracts of *Wedelia chinensis* can be employed in the treatments of diseases caused by the test organisms along with the silver NPs which have wide range applications. Hence, *Wedelia chinensis* can be used to discern bioactive natural products and new pharmaceutical molecules that serve in the development of unmet therapeutic needs.

Keywords: *Wedelia chinensis*, phytochemicals, bioactive compounds, silver nano particles- brine shrimphs, seed germination

Introduction
The plants species are being employed as the first medicine for the human being from ancient world. It has been found that hundreds of plant species are harvested for their medicinal properties all over the World. Medicinal plants still remains an important tool for treating various diseases though modern pharmaceutical chemical drugs. Ethnopharmacology is being integrated into mainstream medicine. Phyto-pharmaceuticals are promising drug candidatures in the current development as well as to expand the biological activity of natural products. The natural product such as *Wedelia Chinensis* plant is a normal ingredient of anti- inflammatory herbal medicines being used in many countries. All parts of the plant possess medicinal applications and have been reported to have beneficial effects on several ailments. It is a perennial herb with bright yellow flowers and a light with bell shaped, camphor-like odor and is used to relieve fever, reduce a cough and phlegm. The medicinal value of the presented plant lies in its chemically active substances and its bioactive constituents are alkaloids, tannin, flavonoid and phenolic compounds [1].
The worldwide dependence on natural agents like plant extracts, as powerful alternatives to chemical and synthetic antimicrobials, is greatly desired by modern consumers. The Antimicrobial and Antifungal plant extracts can be used for various bacterial and fungal pathogen controls. Prevention of pathogenic and spoilage-causing microorganisms in food is usually achieved by using synthetic chemical preservatives with many carcinogenic and teratogenic damages, as well as inherent toxicity, together with growing concern of microbial resistance toward conventional preservatives. For progressive removal of chemical preservatives, natural alternatives are desired to obtain its goals concerning safe food with longer shelf life. Therefore, silver (Ag) nanoparticles that provide protection against bacteria need to be synthesized. Ag nanoparticles can be easily prepared from the plant with non-toxicity in nature for prohibiting the bacteria and fungi, and preventing burns and wound infections [1-3].
The modern therapy in the treatment of diseases like cancer, cardiovascular or rheumatic diseases applies more and more combinatorial approach. High-throughput methods are available to investigate complex mixtures of whole plant extracts and establish synergistic effects for multi-therapeutic target medicines. This approach further helps scientific validation of plant extracts / bioactive molecules that are already being used in traditional medicine [1-3]. The research is aiming and hoping for the introduction of green technology for plant-based products as well as the potential applications of these extracts to improve human health and safety. Furthermore, studies are to be carried out for advanced therapeutic potential which will benefit the mankind.

Materials and Methods
1. Collection of plant
The plants were collected from the sewage damp soil area from Tilak nagar area of Kurla in Mumbai, India. The fresh parts of the plant like leaves, stems and roots were separated and rinsed with distilled water to remove soil dirt. The plant was shade dried for 3-4 days and powdered using electric grinder. The fine leaf powder was transferred into sterile air-tight container and stored for future use.

2. Solvent extraction of plant parts
(a) Extraction of plant parts using methanol solvent:
Extraction of the plant extracts was carried out in a reflux apparatus using 200 gm of dried powder of *W. Chinensis*

whole plant including leaves, stem and roots into capped in round bottom flask contained 500 ml of methanol. The extracts from the plant was carried out for 3 hrs and the solvent was concentrated under reduced pressure at the end of extraction. The obtained crude extract from the plant was stored in refrigerator for further process.

(b) Phytochemical screening

Phytochemical analyses for Alkaloids, Saponins, Phenols, Flavonoids, Tannin, Carbohydrates, Terpenoids, Glycosides, Quinones and Proteins were conducted as per the standard protocols [4, 5].

3. Determination of total phenolic content

The resultant amount of phenolic content was evaluated from Folin-Ciocalteu colorimetric method. The 0.5 ml of Folin-Ciocalteu reagent was reacted with the rest sample and reaction was neutralized with saturated Na_2CO_3 for 1.5 h in the dark at 22ºC. After completion of reaction, the orange color was observed at 660 nm [15]. Consequently, the existence of phenolics in the extracts was computed by calibration curve, which obtained from standard absorbance of Tannic acid standard (100µg/ml) of known concentrations. The presence of total phenolic contents was expressed as Tannic acid equivalence (TAE) in mg.

3.1 Preparation of working stock of the plant extracts

The dried plant powder (50 gm) was dissolved in 100ml distilled water to obtain a concentration of 0.5g/ml plant extracts. This extract (1 ml) was diluted to 10 ml to get the concentration of (0.05g/ml).

3.2 Phosphomolybdate assay for total antioxidant capacity.

The phosphor- molybdenum method was used to analyze the total anti-oxidant capacity (TAC) assay of sample. The sample solution (1ml) was mixed with phosphor-molybdate reagent (2ml) and poured into a test tube. The mixed solution containing test tube was incubated in a water bath at 95^0 C for one and half hours. The 4-20µg/ml concentration range of standard ascorbic acid was employed at a regular interval. After the samples were cooled, the absorbance spectra of the prepared samples were recorded at 660 nm. Blank was carried out using the same process but containing an equal amount of CH_3OH instead the plant sample. TAC was expressed as ascorbic acid equivalence (AAE) in mg/ml according to Ahmed et al [6].

Microorganisms' collection and culture maintenance

Gram negative bacteria such as *E.coli, K.pneumoniae, P.aeruginosa, S.para typhi A, S.para typhi B, P.mirabilis* and Gram positive bacteria such as *S.aureaus, B.subtilis, S.pyogenes, C. diphtheria* were tested in the laboratory. The bacterial cultures were grown on sterile nutrient agar slants at 37°C for 24 h and were maintained at 4°C until further used. Sub culturing was carried out every 4 weeks to maintain their viability.

Synthesis of Ag NPs by wet reduction method

The green synthesis using wet reduction method was used to extract the Ag NPs from plant extract. It is well known that the Ag NPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibration in silver nanoparticles. In *Wedelia*, leaf, stem and root extracts can be easily monitored from the change in the colour of the reaction mixture from yellowish brown to dark brown. The time duration of change in colour varies from plant to plant [7-9].

1. Method for preparation of Ag NPs of the crude extracts (0.4g/ml) by wet reduction

1.1 Synthesis of Ag NPs from the stem extract

1ml of stem extract was added to 9ml of 1mM solution of silver nitrate and stored in dark. Pale yellow solution was obtained.

1.2 Synthesis of Ag NPs from the root extract

1ml of root extract was added to 9ml of 1mM solution of silver nitrate and stored in dark. Pale yellow solution was obtained.

1.3 Synthesis of Ag NPs from the leaf extract

1ml of Leaves extract was added to 9 ml of 1mM solution of silver nitrate and stored in dark. Pale yellow solution was obtained.

Preparation of control: 1% sodium citrate was added drop by drop in 9ml of 1mM silver nitrate solution prepared in distilled water. The solution was heated until the colour changes. Light green colour solution was obtained. This was set to check the reduction of silver ions to nano particles.

2. Separation and purification of Ag NPs from crude matrix

The Ag NPs were separated by centrifugation at 10,000 rpm for 15 min to remove unwanted biological molecules; subsequently the pellet was re-dispersed in sterile double distilled water. The purification of nanoparticles by centrifugation and re-dispersion in sterile ddH_2O was constantly carried out to ensure the better elimination of free entities. The purified pellet was refrigerated.

3. Synthesis of Ag NPs

Green synthesis of Ag NPs was carried out using wet reduction method using 1mM silver nitrate solution. Along with the test samples control was also set with sodium citrate. It is due to the change in the optical properties of Ag NPs as particles aggregated and electrons on surface of each nanoparticle become delocalized. Due to delocalization of the surface electrons, surface plasma resonance (SPR) shifted to lower energies results in the red shift in the absorption and scattering peaks. *Wedelia* synthesized Ag NPs was obtained after 24 hours of incubation. The preparation of Ag NPs indicates that the Ag ions in reaction have been converted to elemental silver of various shape and size. The reduced NPs were stored in dark for 24 hours for further reduction.

Characterization Tools

UV– Vis spectroscopy was used to record absorbance spectra of Ag NPs. The formation of Ag NPs was primarily observed by monitoring the change in colour of the extract after treatment with $AgNO_3$ (1 mM). The bio-reduction of Ag ions in aqueous extract was monitored with the UV–visible spectra of the solutions. Absorbance spectra of the reduced Ag NPs were recorded by UV– Vis spectroscopy (LAMBDA25, Perkin Elmer UV Win Lab) in the range of 200-700 nm at RT. [10].

Results and Discussion

1. Phytochemical Analysis

Phytochemical analysis of the leaf, stem and root extracts was performed and the results are recorded in Table 1.

Table 1: Results of phytochemical analysis of whole plant of *Wedelia chinensis.*

Tests	Results		
	Roots	Leaf	Stem
Alkaloids	+	+	+
Saponins	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Tanins	+	+	-
Carbohydrates	+	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Quinones	+	+	+
Proteins	-	-	-

Table 2: Absorbance of the standard tannic acid in µg/ml at different concentration range

Conc. range of tannic acid (µg/ml)	Absorbance (at 660 nm)
Blank	0.0
20	0.07
40	0.13
60	0.23
80	0.36
100	0.45

From the above Table 2 standard graph of concentration of tannic acid versus absorbance was plotted. As phenolics contain polar phenolic hydroxyl group/s, and their high extraction into methanol and water is quite suitable [6].

The plant was found to be rich in Alkaloids, Saponins, Phenols, Flavanoids, Tanins, Carbohydrates, Terpenoids, Glycosides and Quinones. Alkaloids are organic heterocyclic nitrogen containing compounds, usually with potent activity. Saponins have association with cytotoxic, anti-inflammatory, and anti-tumour activity. Tanins are known to exhibit different biological activities including antibacterial and antifungal activities. Flavanoids are known to possess antioxidant, anti-inflammatory and anti- tumour activity. Of particular importance terpenoids have antineoplastic, antibacterial, antiviral effects as well as gastrointestinal stimulations. Glycosides serve in the inhibition of Na+/K+-ATPase-pumps in the cell membranes. Quinones contain Naphthoquinone group which has a wide range of biological activities. Thus, the presence of all these compounds is indicative of the biological activity of the whole plant of *Wedelia chinensis* [11].

2. Estimation of total phenolic content

The total phenolic content of the plant extracts including leaf, stem, and roots was estimated using Folin ciocalteau method. The absorbance was recorded at 660 nm. Using the standard plot, the concentration of phenolic contents in the leaf, stem and root extracts were estimated. The concentration of phenol in the plant extracts (0.5g/ml) was calculated as Tannic acid equivalents (TAE). It was found that all the three plant extracts contained good amount of phenolic content ranging between 600 to 800 µg/ml or tannic acid equivalents. But the concentration of the plant extracts used for the test was 0.5g/ml. hence the concentration of phenol in 1mg/ml is to be determined followed by concentration of phenol in 1ml in µg of TA which is clearly calculated in the Table 3.

Table 3: Result of plant extracts (0.5g/ml) for total phenolic contents in mg of TAE and total anti-oxidants in mg of AAE.

Test sample	O.D at 660nms for total phenolics	Conc. of phenol in 1g/ml of the sample in µg/ml of TA	Conc. of phenol per ml of sample in mg of TAE	OD at 660nms for total anti- oxidants	Conc. of AO in 1g/ml sample in µg/ml of AA	Conc. of AO per ml in mg of AAE
Leaf	0.25	1208	1.208	0.17	171.4	0.17
Stem	0.33	1526	1.526	0.33	273	0.27
Roots	0.28	1326	1.326	0.45	349.2	0.34

3. Phosphomolybdate assay for total antioxidant capacity.

Using the above standard range the anti-oxidant capacity was determined as ascorbic acid equivalence which is as shown in the Table 3. The concentration of anti-oxidants in the plant extracts (0.5g/ml) was calculated as ascorbic acid equivalents (AAE). It was found that all the three plant extracts contained good amount of anti- oxidants ranging between 0.10 to 0.40 µg of ascorbic acid equivalents. But the concentration of the plant extracts used for the test was 0.5g/ml. Hence the concentration of anti-oxidants in 1g/ml is to be determined followed by concentration of anti-oxidants in 1ml of extract in µg of AAE which is clearly calculated in the table below.

From the above readings calibration curve of concentration range of standard ascorbic acid versus absorbance was plotted. A linear plot was obtained from the above readings. Using this standard plot the concentration of the anti-oxidants in the test sample was determined as in the Table 3.

4. Antimicrobial activity of plant extract

The well diffusion method was used to obtain the antibacterial activity of the plant extracts against the test bacteria. Test bacteria were cultured on nutrient agar plates and incubated at 37° for 24 h. Bacterial suspensions were prepared by inoculating one loopful of a pure colony into 2.0 ml of sterile distilled water (SDW). Sufficient inoculum was added until the turbidity matched with 0.5 McFarland standards which was approximately equivalent to 1.5×10^5 cells/ml. Initially the concentration of the leaf, stem and root extracts was employed as 50g, 100g, 150g and 200g of dried plant powders [15]. But, only 200g conc. showed significant antimicrobial potential.

Therefore the test was done with 200g (0.4g/ml) plant powder in triplicates for result confirmation. 1ml inoculum suspension was swabbed on the surface of sterile nutrient agar plates using sterile cotton swabs for uniform distribution of bacteria. Subsequently, using sterilized borer wells of 0.5 cm were made in the inoculated media plates. 0.3 ml of the extract (0.4g/ml) was added aseptically in the well. The plates were placed at RT for an hour to allow diffusion of extract into the agar. The plates were then incubated at 37° C for 24 h and the antibacterial activity was determined by measuring the diameter of the inhibition zones formed around the disks [15].

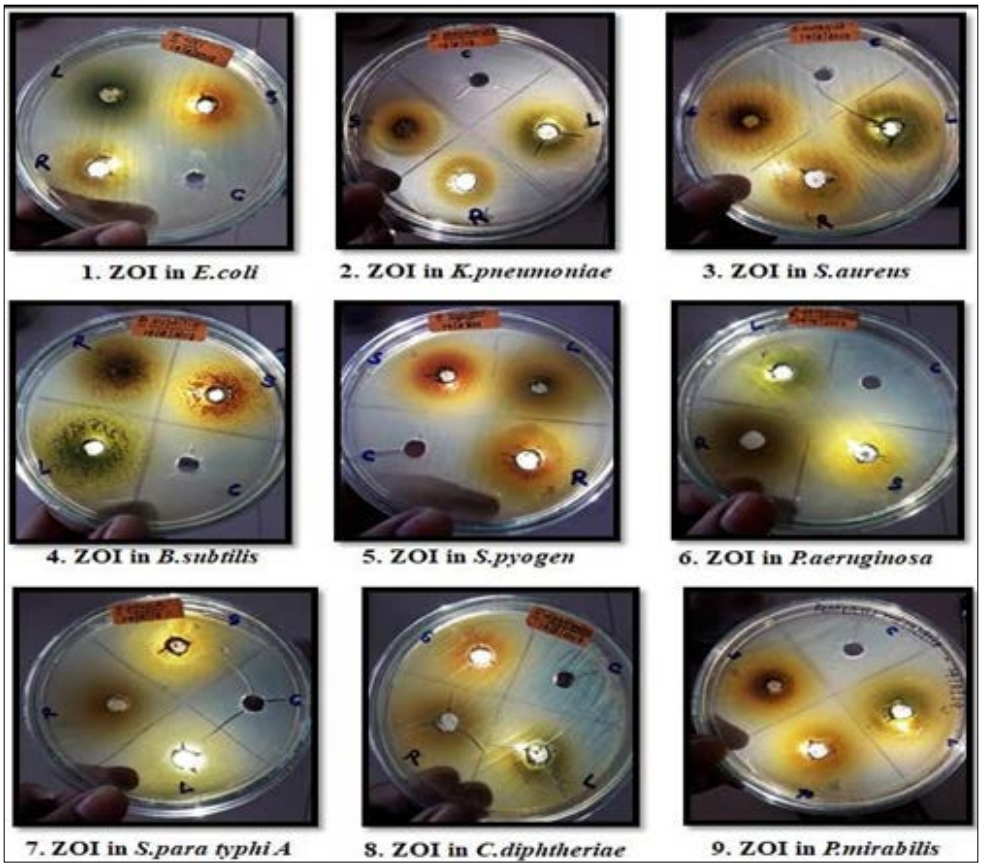

Fig 1: Antimicrobial activity of the plant extracts (0.4g/ml) against test organisms

Control: Along with the test a control was also set to check the presence of traces of methanol in plant extracts which could lead to microbial inhibition. For this 10 ml of methanol was allowed to evaporate overnight. The empty petri plate after evaporation was washed with 10 ml distilled water which was used as control to check antibacterial activity.

5. Synergistic Activity of plant extract

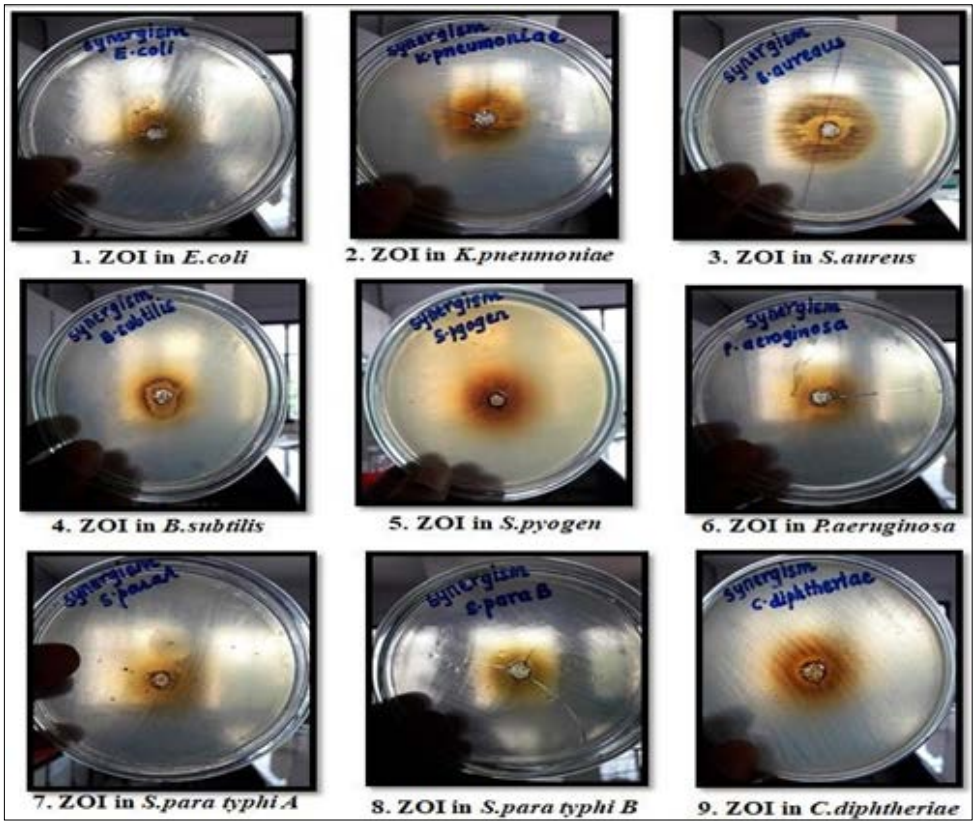

Fig 2: Synergistic activity of plant extracts (0.4g/ml) against test organisms.

The synergistic activity of the extracts of *W. Chinensis* was investigated against the bacteria cultures such as gram negative (*E.coli, K.pneumoniae, P.aeruginosa, S.para typhi A, S.para typhi B, P.mirabilis*) and gram positive (*S.aureaus, B.subtilis, S.pyogens, C.diphtheriae*) respectively. The inoculums of lag phase culture of microorganisms were prepared from bacterial cultures by McFarland standard. The 1ml inoculum suspension using sterile was used on the surface of sterile nutrient agar plates for homogeneous distribution of bacteria. Subsequently, the sterilized borer wells of 0.5cm diameter were produced in the inoculated media using a sterile nutrient agar plate using sterile cotton swabs. The 0.3 ml of extract mixture (0.1ml of leaf, stem, and root 0.4g/ml) was mixed together in the ratio of (1:1:1) and filled into the well. For the diffusion of extracts into the agar, the plates were placed at RT for 1h and incubated for 24h at 37°C. The obtained outcomes were recorded by the diameter of inhibition zone.

Control: Along with the test a control was also set to check the presence of traces of methanol in plant extracts which could lead to microbial inhibition. For this 10 ml of methanol was allowed to evaporate overnight. The empty petri plate after evaporation was washed with 10 ml distilled water which was used as control to check antibacterial activity.

6. Antimicrobial activity of crude extracts

The antibacterial efficacy of the crude extracts of *Wedelia chinensis* was evaluated using well diffusion method against ten cultures. The antimicrobial activity of the leaf, stem and root extracts is presented in the Table 4. *S. Pyogens* and *S.Para typhi B* exhibits the absence of any significant inhibition of their growth in the presence of the three extracts. The test was done in triplicates for consistent results.

Table 4: Antibacterial and Synergistic zone of inhibition on exposure 0.4 g/ml concentration of Crude extracts.

Test organism	Zone of inhibition (mm)				Zone of inhibition (mm)	
	Root extract	Leaf Extract	Stem extract	Control	Synergistic activity	Control
E.coli	12	14	13	-	14	-
K.pneumoniae	13	12	11	-	17	-
S.aureus	13	15	17	-	18	-
B.subtilis	14	16	12	-	17	-
S.pyogens	-	-	-	-	14	-
P.aeruginosa	16	18	17	-	14	-
S.para typhi A	15	14	15	-	13	-
S.para typhi B	-	-	-	-	12	-
C.diphtheriae	12	14	13	-	14	-
P.mirabilis	14	18	16	-	12	-

7. UV Absorption peak for leaf extract based Ag NPs

For the Ag NPs prepared from leaf extract the scanning absorption range was employed from 200 to 700 nm. Three peaks were obtained at 237, 298 and 358 nm as indicated in Fig.3. This indicates that the peak around 358 nm is attributed to SPR band of the leaf Ag NPs, whereas peaks below 300 nm indicate blue shift which may be due to impurities, organic species, solvent etc. Moreover, the broad peak at 358 nm indicates the decreased particle size according to the quantum size confinement [12].

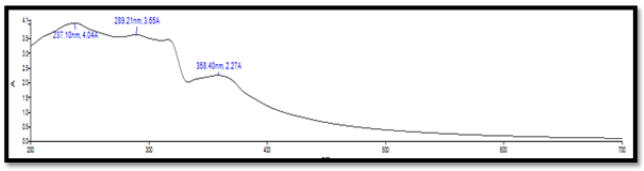

Fig 3: UV Absorption peak for leaf extract based Ag NPs

8. UV Absorption peak for stem extract based NPs

The stem based Ag NPs exhibited five peaks 239, 259, 290, 308, and 392 nm (Fig.4). The peak near 392 indicates strong surface plasmon resonance band. The other peaks below 300 indicate blue shift which may be due to impurities, organic species, solvent etc. The peak is broad at 392 which confirm the presence of decreased particle size according to the quantum size theories [12].

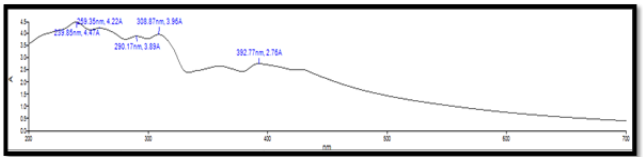

Fig 4: UV Absorption peak for stem extract based Ag NPs.

9. UV Absorption peak for Root extract based Ag NPs

Fig. 5 exhibits the sharp absorption peaks at 417 nm and 431nm indicates the formation of Ag NPs. The SPR band shows broad peak at 417 nm indicating decreased particle size and the peak at 431nm indicates the increased particle size according to the quantum size theories [12].

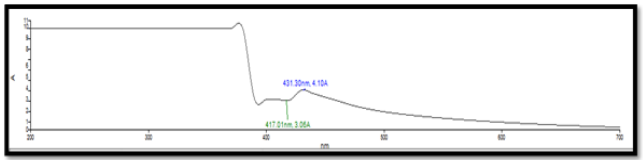

Fig 5: UV Absorption peak for root extract based Ag NPs.

10. Antimicrobial activity of Ag NPs

The antibacterial activities of the synthesized Ag NPs were tested against test bacterial cultures using well diffusion method. 1ml each of test bacterial cultures (0.5 McFarland standards) using sterile was used on the surface of sterile nutrient agar plates for homogeneous distribution of bacteria. Subsequently, sterilized borer wells of 0.5 cm diameter were produced in the inoculated media and then after, 0.3 ml of the Ag NPs solution was added aseptically in the well. The plates were placed at RT for an hour to allow diffusion of extract into the agar. The plates were then incubated at 37°C for 24 h and the antibacterial activity was determined by measuring the diameter of the inhibition zones formed around the wells [16, 17].

Ag NPs were produced and their efficacies as antimicrobials were tested using ten bacterial cultures by well diffusion method. The results of the antimicrobial activity of the Ag NPs were recorded as in Table 5. It was found that Ag NPs were really efficient for inhibiting growth of the bacterial cultures. Along with the three Ag NPs (leaf, stem, root) control also showed significant result indicating the proper bioreduction of the silver nitrates to silver nanoparticles of the plant extracts. The SNPs synthesized from plant species are toxic to many microorganisms. From this study it was clear that they have great potential in biomedical

applications. For consistent results the test was done in duplicates and same results were obtained [16, 17].

Table 5: Antibacterial zone of inhibition on exposure of Ag NPs of different extracts.

Test organism	Zone of inhibition (mm)			
	Root extract	Leaf Extract	Stem extract	Control
E.coli	26	17	19	25
K.pneumoniae	20	19	18	18
S.aureus	38	18	20	30
B.subtilis	28	14	11	23
S.pyogen	20	15	24	18
P.aeruginosa	22	16	17	19
S.para typhi A	28	20	25	30
S.para typhi B	30	19	14	24
C.diphtheriae	30	14	16	26
P.mirabilis	26	18	17	25

11. Brine shrimph lethality assay.

Cyto-toxicity test of plant extracts and silver nano particles was studied using brine shrimph lethality assay. The motility of the hatched naupalli's was recorded upto 24 hours (Fig. 6).

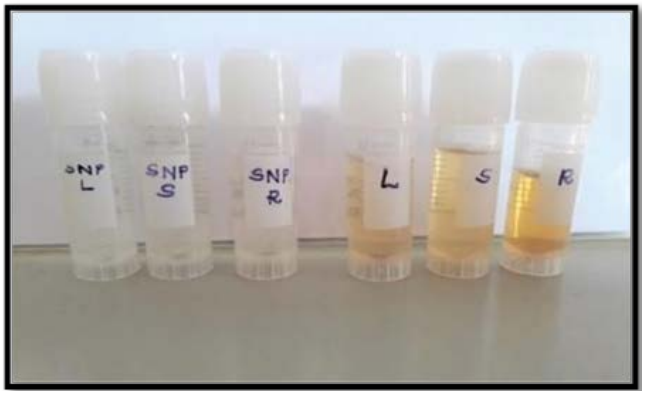

Fig. 6: Brine shrimph lethality assay with plant extracts.

11.1 Cyto-toxicity of plant extracts

In this test 1ml hatched larvae (each containing 10 larvae) was mixed with 0.05ml of leaf, stem and root extracts. The motility of the larvae was observed only upto 8hours. No motility was seen after 8 hours which may be because of the presence of plant phytochemicals as the larvae are very small these phytochemicals have the property to directly accumulate in their gut leading to death. Significant cyto-toxic activity could be due to the presence of saponins which are well known to possess such cyto-toxic potential.

11.2 Cyto-toxicity of Ag NPs

In this test 1ml hatched larvae (each containing 10 larvae) was mixed with 0.05ml of Ag NPs prepared from leaf, stem and root extracts. The motility of larvae in Ag NPs was observed upto 24 hours. The larvae were actively motile upto 24 hrs indicating that the Ag NPs are not toxic as it do not cause any potential harm to the larvae.

Applications of Ag NPs

1. Toxicity test by brine shrimph lethality assay

Brine shrimphs eggs were purchased from local market. 0.1 gram of eggs were weighed and added in a flask containing 100ml distilled water and 2.5 grams of black salt. The flask was incubated on a shaker for 24 hours and was further incubated for the next 48 hours at room temperature. After 3 days of incubation the eggs were hatched into naupalli's. The test involved 1ml of hatched naupalli's (each tube containing 10 larvae) and 0.05ml of the plant extracts (0.4g/ml). Same protocol was used for silver nano particles where 1ml of the larvae (each tube containing 10 larvae) was mixed with 0.05ml of silver nano particles prepared from plant extracts. Motility was observed till 24 hours and the mortality rate was determined [13].

2. Effect of green synthesized Ag NPs on seed germination

The effect of Ag NPs on seed germination, root-shoot length along with some physiological parameters in leaves and roots of Triticum (wheat plant) was analyzed by pot study. Seeds were sowed in sterile soil in a clean petri plate. 10ml sample of each Ag NPs was added to the respective petri plate. To study the synergistic activity of the Ag NPs on seed 10 ml of each 1mM silver nano solution made from leaf, stem, roots was added to the different petri plates containing seeds. A control was also set with seeds and water to compare the results. The results were recorded upto 5 days**.**

Fig 7: Seed germination for 5 days

The pot study was successful in evaluating the effect of Ag NPs on the seed germination of wheat plant. After 24 hrs seed were germinated. The results were recorded after five days of incubation (Fig. 7). It was observed that the hand sanitizer prepared from Ag NPs of plant extracts was very efficient in reducing the microbial count upon use. Along with this hand prints were also taken on sterile nutrient agar plate to test qualitatively the efficiency of the hand sanitizers. On comparing the results of before and after use tests it was observed. Upon comparing the results with the control (Seeds germinated in water) it was observed that Ag NPs are greatly efficient in inducing seed germination process. Maximum growth was observed for individual Ag NPs made from leaf, stem and roots. The test containing the mixture of all the three Ag NPs did not showed significant result. Hence it can be said that Ag NPs solutions were able to penetrate the seed coat into endosperm and embryonic tissues which increased the water uptake efficiency resulting in faster seed germination process [14, 18]. Also the results of the spread plate count were compared with commercial hand sanitizer containing chemical disinfectants and it was found that this silver containing sanitizer were equally effective being an herbal formulation (Fig. 8).

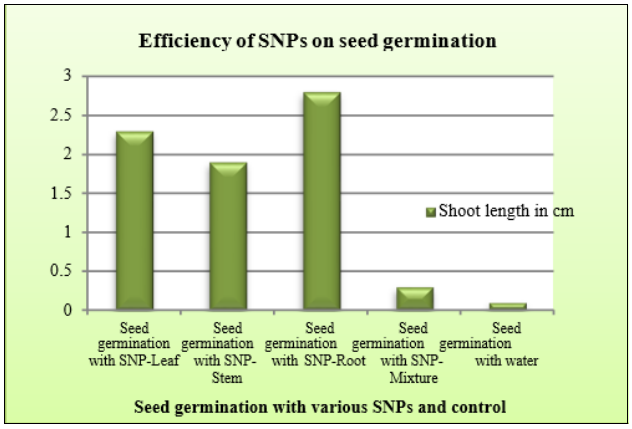

Fig 8: Graph of comparison of efficiency of SNP on seed germination.

Conclusions

From the study it can be concluded that *W. Chinensis* possess significant amount of phytochemicals which contributes to antimicrobial potential against the test organisms. The total phenolic content and total anti-oxidants in the plant extracts were also estimated and it was found that the plant contains good amounts of phenolics and anti-oxidants which can be used in nutraceuticals for therapeutic use. The antimicrobial activity of *W. Chinensis* whole plant extracts studied by well diffusion method showed its effective antimicrobial potential against the test organisms. Synergistic potential of leaf, stem and root extract was also studied by well diffusion method against the same bacterial cultures and all the cultures were found to be susceptible. Ag NPs synthesized from the plant extracts showed good antimicrobial activity. This suggests that the extracts and the Ag NPs can be used in treating diseases caused by the test organisms. Characterization of these green synthesized Ag NPs by UV- Vis spectroscopy confirmed the synthesis of Ag NPs with their corresponding SPR bands. Cyto-toxicity of the plant extracts and Ag NPs determined by brine shrimph lethality assay showed that Ag NPs have low mortality rate as compared to plant extracts as plant extracts tend to accumulate in their gut leading to death. Owing to the effective antimicrobial potential of SNPs' they were formulated into a alcohol free sanitizer where they were found to be very efficient in reducing microbial counts from hands. These SNP's were also found to be efficient in germination of *Triticum* seeds (wheat). Hence these results suggest that the Ag NPs have wide range of applications from antimicrobials to plant growth promoters which aids in rapid plant growth. Hence, *W. Chinensis* can be used to discern bioactive natural products by introducing phyto-pharmaceutical molecules that will serve for human welfare.

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