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COMPARISON OFANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF CRUDE PLANT EXTRACTS WITH THEIR SYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT

Research and analysis of nanoparticles (NPs) synthesis with plant extracts and their biological activities has been expanded significantly in the recent years. Among the agents used for synthesis, silver (Ag) is the most preferred due to its reported use in medical field as best topical bactericides from ancient times. In the present study, crude bark extracts of Acacia auriculiformis A. Cunn. were used for nanoparticles synthesis and comparison of antimicrobial activity of crude extract with NPs and commercial antibiotics was also assessed against Bacillus subtilis (B1) and Escherchia coli (B2). Also the synthesized NPs and crude extracts were compared for their antioxidant potential.

1. INTRODUCTION

Research and analysis of nanoparticles (NPs) synthesis and their biological activities has been expanded significantly in the recent years. The agents used for nanoparticles (NPs) synthesis are of organic (mainly carbon) and inorganic (metal ions like silver and gold) origin [1]. Among these, silver (Ag) is the most preferred NPs synthesis agent due to its reported use in medical field as best topical bactericides from ancient times [2]. The stable silver nanoparticles had been synthesized by using soluble starch as both the reducing and stabilizing agents [3]. So the concern of scientific community shifted towards ecofriendly, natural and cheaper method of NPs synthesis by using microorganisms and plant extracts [4]. The use of plant materials for silver nanoparticles (NPs) is most popular due to its potential biological activities, easy availability and faster rate of synthesis there by cutting the cost of NP's synthesis [5,6]. The nanoparticles had been clinically used for infection, vaccines and renal diseases [7]. The plant extract of petals of herbal species like *Punica granatum*, *Datura metel* and Aloe vera [8] and stem extracts of *Svensonia hyderobaden*sis [9] had been effectively used for NPs synthesis and investigated for their antimicrobial activities.

Nanoparticles could be synthesized by various approaches like photochemical reactions in reverse micelles, thermal decomposition, sonochemical and microwave assisted process [10, 11]. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics [12], antimicrobials and therapeutics [13, 14] and micro-electronics [15].

A. auriculiformis (also called as Black wattle and Australian Kikkar) is an important medicinal plant and widely distributed member of family Fabaceae and subfamily Mimosoideae. It had reported to be a rich source of polyphenols and tannins [16]. It's anti-helminthic, anti-filarial and microbicidal effects had been well demonstrated [17]. Extensive literature and research of the medicinal as well as biological properties of *A. auriculiformis* at cellular stage [18], drug delivery and diagnostics imaging cancer detection [19] were

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available in the reports of many scientists.

There are no reports available for silver nanoparticles (NPs) synthesis using bioactive extracts of important medicinal plant, *A. auriculiformis*. In the present study, antimicrobial and antioxidant activity of crude extracts (methanol (AAM), acetone (AAA) and water (AAW) extract) of *A. auriculiformis* as well as synthesized nanoparticles was explored.

2. MATERIALS AND METHODS

The bark of *A. auriculiformis* was collected from Chandigarh Sec-34 along the road side. The plant species was identified by comparing it with the specimen available in the herbarium (voucher number 6422) of the Guru Nanak Dev University, Amritsar. Utmost care was taken to select the healthy bark.

Two strains of bacteria *Bacillus subtilis* (B_2) (MTCC No. 10619) and *Escherchia coli* (B_1) (MTCC No. 1652) were used for investigating the antimicrobial activity of *A. auriculiformis* crude bark extracts and NPs. The cultures were obtained from Department of Microbiology, Dolphin (PG) College of science and agriculture, Chunni. The cultures were maintained on nutrient agar at 4^0 Celsius.

2.1. Culture media

Nutrient broth: Nutrient broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biologically fluid.

Muller hinton agar: It is a microbial growth medium that is commonly used for antibiotic susceptibility testing.

Table 1 Composition of Nutrient Broth (NB) used for culturing of bacterial strains

S. No	Chemicals	Amount
1.	Peptone	5.0gm
2.	Beef extract	3.0gm
3.	Sodium chloride	5.0gm
4.	Distilled water	1000ml

Table 2 Composition of Muller Hinton Agar (wt/vol.) used for culturing of bacterial strains

S. No	Chemicals	Amount (%)
1.	Beef infusion	30
2.	Casien hydrolysate	1.75
3.	Starch	0.15
4.	Agar	1.7

The above listed ingredients were carefully weighed, dissolved in measured amount of distilled water and after setting their pH to 6.8-7.2, the containers were properly plugged and sterilized by autoclaving.

Maintenance and preservation of cultures: The strain of bacteria was maintained on nutrient Agar at 4^0C in laboratories of Department of Biotechnology, Dolphin (PG) College of Science and Agriculture and sub-cultured bimonthly for routine use.

2.2. Preparation of bark extract

Procurement and extraction of plant material: The bark of *A. auriculiformis* was procured and washed with tap water (thrice) and dried in oven at 30°c for overnight and ground to a fine powder with grinder. The extracts of *A. auriculiformis* were prepared by maceration extraction method in which the bark was dissolved in solvent.

***Protocol for extraction of plant material*:** Dried bark powder (3Kg) was suspended in 1000ml methanol and kept on shaker for 24hr at room temperature. After 24hr, the suspended solid was filtered off through Whatman No. 1 filter paper and filtrate was collected.

This procedure was repeated thrice to obtain three filtrate of extract. The three filtrates were combined and solvent was removed under vacuum using rotary evaporator to obtain solid residue.

This light brown coloured dried material was named as "Methanol Extract" of *A. auriculiformis* (AAM). Other extracts i.e. acetone (AAA) and water (AAW) were also obtained with the similar method.

Preparation of stock solutions of extracts:

(1) Prepare 7% of di-methyl sulphoxide by adding 7ml of DMSO into 93ml of Water
(2) Dissolve 10mg of extract into 1ml of 7%DMSO for T_1 conc.
(3) Dissolve 5mg of extract into 1ml of 7%DMSO for T_2 conc.
(4) Dissolve 2.5mg of extract into 1ml of 7%DMSO for T_3 conc.
(5) Dissolve 1.25mg of extract into 1ml of 7%DMSO for T_4 conc.
(6) Take 1ml of 7% DMSO as control.
(7) Then assessed the microbial activity of extracts as well as control.

2.3. Antibacterial activity of crude extracts

Agar well diffusion method:The antibacterial activity was assessed using the simple agar well diffusion method. The extract was poured in wells made on nutrient media inoculated with the test bacteria so as to get a lawn culture on incubation. The extract diffuses into the medium and inhibits bacterial growth around the well which indicates its antibacterial efficacy. Larger is the zone of inhibition (ZOI) higher will be the antibacterial activity.

Petri plates were prepared by pouring about 25ml of Muller Hinton Agar in each plate for different bacterial samples. Control was designated as C. Plates were dried and 100µl of inoculum suspension was poured and then spread uniformly on the agar plate. The access inoculum was drained and then allowed to dry for 5min. Four wells of size 6mm diameter were formed in each petri plate (one at middle and three on sides of agar plates). 20 µl of extract was poured in the wells of respective petri plates. The plates were incubated in shaker incubator at 37^0C for 24hrs. The ZOI was measured after 24hrs of incubation by measuring the average diameter of wells marked as (B_1, B_2 and C) of all the petri plates.

2.4. Protocol for synthesis of nanoparticles

1. Take finely powdered extract of the plant.
2. Prepare the stock solution of the plant material with 10mg/ml conc. of extract in 100ml of 7% DMSO.
3. Prepare 1mM $AgNO_3$ (silver nitrate) solution.
4. Add $AgNo_3$ solution to extract solution in 9:1.
5. Mix 900ml of $AgNO_3$ solution to 100ml of plant extracts of test conc.
6. Incubate the solution for 2-3 hrs and filter it.
7. Dry the solution in hot air oven 40-50^0 C.
8. Grind the dried material into fine powder.
9. Wash the powder with NaCl (sodium chloride). If white precipitates formed, filter it using Whatman filter paper.
10. Dry it and powdered the material.
11. Store the material for further experimentation in air tight containers.

Preparing stock solution of NPs for anti-microbial activity: Mix 10mg/ml conc. Stock solution of NPs for anti-microbial activity assay by taking Cloramphenicol as a reference antibiotic.

2.5. Antibacterial activity of synthesized silver nanoparticles

The antibacterial activity was assessed by agar well diffusion method which was explained as antibacterial activity of crude extracts.

2.6. Antioxidant activity

Total phenolic content **(TPC):** The total phenolic content in methanol extract was observed using modified method of Lister and Wilton [20].

Ascorbic acid (vitamin –C): Ascorbic acid in extract was estimated by indo phenol titration method [21].

Hydrogen peroxide scavenging activity assay: The methodology of hydrogen peroxide scavenging activity assay was performed with guidelines explained by Klein and co-workers [22].

Ferric thiocyanate assay **(FTC):** The antioxidant activity of extract was tested using the Ferric Thiocyanate assay method following the standard method given by Mistuda and Yusasumoto in 1996 [23] along with other scientists.

2.7. Statistical analysis

The data was computed and statistically analyzed by using SPSS and ASSISTAT computer software and the statistical tests (Analysis of variance (ANOVA), Standard error (SE) and Tukey's Test employed according to the requirements of experiments.

3. RESULTS AND DISCUSSION

3.1. Effect of crude plant extracts on B1 (*Bacillus subtilis*)

AAW: ZOI was comparable with antibiotic treatment and crude extract treatments. In control (C), minimum (0.56 cm) ZOI is recorded whereas a significant decrease was recorded from first treatment level (T1) to third treatment level (T3) with a slight increase in fourth treatment level (T4) (Table 3).

AAM: Methanol extract showed consistent and significant decrease in ZOI from C to T1 and from T1 to T4. Maximum ZOI was recorded with antibiotic treatment (Table 3).

AAA: In contrast to AAW and AAM, AAA showed a continuous and significant increase in ZOI from C to T4 (Table 3).

3.2. Effect of crude plant extracts on B2 (*Escherichia coli*)

AAW: ZOI significantly increased from 0.54cm to 1.50 cm in T1 and it is almost comparable in other treatment levels. Highest ZOI (1.70 cm) was recorded with T2 (Table 3).

AAM: With methanol extract treatment ZOI showed decrease from C to T2, then value increases in T3 and again decreases in T4. The changes in ZOI were non-significant with AAM treatment (Table 3).

AAA: It showed irregular changes in ZOI with maximum value in T1 and minimum in C i.e. 1.43cm and 1.27cm respectively (Table 3).

The inhibitory effect of *A. auriculiformis* was comparable with the earlier results [24]. The mean diameter of inhibition zones obtained with *B. subtilis*, *Streptococcus* sp., *E. coli* and *P. aeruginosa*. The NPs can bind to G^-cell wall better and the synthesized NPs of crude extract produce equal sensitivities toward *E. coli* and *P. aeruginosa.*

Table 3 Antibacterial activity in the form of Zone of Inhibition (ZOI) of *Acacia auriculiformis* extracts against B1 (*Bacillus subtilis*) and B2 (*Escherichia coli*)

	AAW (Mean±SE)		AAM (Mean±SE)		AAA (Mean±SE)	
	B1	B2	B1	B2	B1	B2
	Zone of Inhibition (cm)					
Antibiotic	1.80 ± 0.21^{a}	1.60 ± 0.17^{a}	3.00 ± 0.12^{a}	2.80 ± 0.12^{a}	3.07 ± 0.12^{a}	2.70 ± 0.11^{a}
Control	0.56 ± 0.11^{a}	0.54 ± 0.88^{a}	1.43 ± 0.12^{a}	1.53 ± 0.09^{a}	1.24 ± 0.12^{a}	1.27 ± 0.06^{b}
T1	1.50 ± 0.05^{a}	1.50 ± 0.05^{ab}	1.90 ± 0.05^{b}	1.46 ± 0.06^{a}	1.20 ± 0.06^{b}	1.43 ± 0.06^{b}
T2	1.21 ± 0.04^{a}	1.70 ± 0.27^{a}	1.27 ± 0.01^{ab}	1.36 ± 0.06^{a}	1.30 ± 0.06^{b}	1.42 ± 0.06^{b}
T3	1.23 ± 0.06^{a}	1.50 ± 0.26^{ab}	1.26 ± 0.10^{ab}	1.50 ± 0.12^{a}	1.43 ± 0.03^{b}	1.33 ± 0.06^{b}
T4	1.40 ± 0.11^{a}	1.53 ± 0.24^{ab}	1.22 ± 0.08^{b}	1.40 ± 0.06^{a}	1.47 ± 0.03^{b}	1.33 ± 0.07^{b}
F-Value	2.7028^{*}	3.8064^{*}	3.1813^{*}	0.4479^{ns}	9.3125^{**}	24.3103^{**}

*** Significant at a level of 1% of probability (p < .01)*
** Significant at a level of 5% of probability (.01 =< p < .05)*
ns Non-Significant (p >= .05)

3.3. Effect of nanoparticles (NPs) synthesized from plant extracts on B1 (*Bacillus subtilis*)

AAW-NPs: ZOI showed slight increase from 0.40cm to 0.43cm with NPs treatment (Table 4).

AAM-NPs: A significant and notable increase in ZOI was recorded with NPs as compared to Control (Table 4).

AAA-NPs: Treatment with NPs increased ZOI to almost double from its value in control (Table 4).

3.4. Effect of nanoparticles (NPs) synthesized from plant extracts on B2 (*Escherichia coli*)

AAW-NPs: ZOI doubled in NPs treatment as compared to control (Table 4).

AAM-NPs: Fourfold increase in ZOI value was recorded from control to NPs treatment (Table 4).

AAA-NPs: ZOI increased significantly from 0.40cm to 1.50cm in C and NPs treatment respectively (Table 4).

Table 4 Antibacterial activity in the form of Zone of Inhibition (ZOI) of silver nanoparticles synthesized with *Acacia auriculiformis* extracts against B1 (*Bacillus subtilis*) and B2 (*Escherichia coli*)

Treatment	AAW (Mean±SE)		AAM (Mean±SE)		AAA (Mean±SE)	
	B1	B2	B1	B2	B1	B2
	Zone of Inhibition (cm)					
Antibiotic	1.80 ± 0.24^{a}	1.60 ± 0.17^{a}	3.00 ± 0.09^{a}	2.80 ± 0.12^{a}	3.07 ± 0.09^{a}	2.70 ± 0.12^{a}
Control	0.40 ± 0.11^{b}	0.20 ± 0.08^{b}	0.70 ± 0.12^{c}	0.40 ± 0.05^{c}	0.70 ± 0.05^{c}	0.40 ± 0.12^{c}
NPs	0.43 ± 0.03^{b}	0.40 ± 0.05^{b}	1.60 ± 0.15^{b}	1.60 ± 0.09^{b}	1.43 ± 0.10^{b}	1.50 ± 0.16^{b}
F- value	29.123**	36.6364**	134.333**	108.00**	104.2895^{**}	70.0580^{**}

** Significant at a level of 1% of probability ($p < .01$)
* Significant at a level of 5% of probability ($.01 =< p < .05$)
^{ns} Non-Significant ($p >= .05$)

The NPs had higher ZOI than crude extracts and increased conc. of NPs had increase the inhibitory effect. The potent inhibitory effect of *A. auriculiformis* was comparable with the earlier results [25] reported the enhanced effects of NPs synthesized from garlic and onion against *Bacillus, Staphylococcus* and *E. coli*.

3.5. Effect of crude extracts and respective nanoparticles on total phenolic content (TPC), Ascorbic acid scavenging activity, Hydrogen peroxide scavenging activity and Ferric Thiocynate scavenging activity

All the three NPs synthesized from their respective crude extracts (AAW, AAM and AAA) showed increased amount of total phenolic content as compared to crude extracts (Table 5.). Similarly, Ascorbic acid scavenging activity, Hydrogen peroxide scavenging activity and Ferric Thiocynate scavenging activity increased in case of NPs when compared with their respective crude plant extracts (Table 5).

Table 5 Antioxidant activity, Ascorbic acid scavenging activity, Hydrogen peroxide scavenging activity and Ferric Thiocynate scavenging activity of extracts and nanoparticles

		Total phenolic content (mg AAM/ml)	Ascorbic acid scavenging activity (%inhibition) = (1-A1/A0)100 (Mean ± SE)	Hydrogen peroxide scavenging activity (%inhibition) = (1-A1/A0)100 (Mean ± SE)	Ferric Thiocynate scavenging activity (% inhibition) = ([A0-A1)/ A0]*100) (Mean ± SE)
AAW	Extract	0.118	80.51 ± 0.01	39.42 ± 0.05	73.08 ± 0.12
	NPs	0.243	88.20 ± 0.03	44.10 ± 0.22	80.02 ± 0.07
AAM	Extract	0.444	82.51 ± 0.04	39.42 ± 0.05	73.08 ± 0.12
	NPs	0.847	90.20 ± 0.03	44.10 ± 0.22	80.02 ± 0.07
AAA	Extract	0.709	78.34± 0.01	44.59± 0.02	79.05± 0.03
	NPs	0.907	80.52± 0.01	63.51± 0.01	88.43± 0.02

4. CONCLUSION

It is clear that NPs of *A. auriculiformis* had much more antioxidant potential then that of crude extracts. There is a direct relationship between antioxidant activity and phenolic content of plant extracts [26]. There are evidences that antioxidants may be useful in preventing the deleterious consequence of oxidation processes and so, the importance of natural antioxidants is increasing day by day. Therefore NPs of *A. auriculiformis* may be trusted for good antioxidant potential.

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