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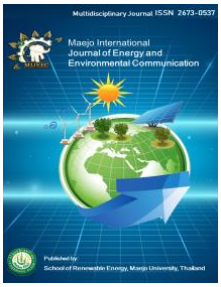

ARTICLE

Sustainable synthesis of silver nanoparticles from *Canna edulis* for eco-friendly applications and their phytochemical and antimicrobial assessment

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ABSTRACT

The increasing demand for eco-friendly and sustainable methods in nanotechnology has shifted focus towards biological routes for synthesizing nanoparticles. This study highlights the green synthesis of silver nanoparticles (AgNPs) using the plant extract of *Canna edulis*, a process that avoids the use of hazardous chemicals. The synthesized AgNPs were characterized for their phytochemical content and evaluated for their antimicrobial properties. *Canna edulis* is an erect herbaceous perennial plant producing clumps of stems with large leaves. The present research was done on *Canna edulis*, where silver nanoparticles were prepared from the extract of leaves and petals. The leaf extracts showed a greater zone of inhibition against E. coli (18±2mm) followed by *Salmonella* spp. and *Klebsiella* spp. (17±1mm), then *Pseudomonas* spp. and *Staphylococcus aureus* (15±1mm). The petal extract of *Canna edulis* was more effective against *Salmonella spp*. and *Pseudomonas spp*. with a 15±3mm zone of inhibition followed by *Staphylococcus aureus* and *Klebsiella* spp. (14±2mm) and the *E. coli* (12±2mm). The characterization of nanoparticles of the extract showed the optimum density from which the maximum peak was obtained at 400nm for leaf and 420nm for the petal respectively. The phytochemical analysis of the leaf extract of *Canna edulis* concluded that alkaloids were present in good concentration whereas in petal extract flavonoids and phenolic compounds gave strong positive results.

1. Introduction

Nanoparticles, especially silver nanoparticles, have garnered significant attention due to their unique properties and potential applications in various fields (Bhuyar et al., 2020; Vatcharakajon et al., 2023). However, conventional synthesis methods often employ toxic chemicals, raising environmental concerns. In this context, green

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synthesis utilizing plant extracts has emerged as an eco-friendly alternative. *Canna edulis*, known for its rich phytochemical content, is explored in this study for its potential in synthesizing AgNPs (Nithin et al., 2023; Dogra et al., 2023).

Canna edulis, also known as Ker-Gawl, is a perennial plant with a starchy rhizome that thrives in the North eastern Himalayan regions of India (Tanaka, 2004). This upright herbaceous perennial grows in clusters, reaching heights of up to 3 meters. Its sizeable leaves, stretching up to 60cm in length and 18cm in width, contribute to its distinctive appearance. Notably, various parts of the plant, including its rhizomes, leaves, flowers, and seeds, possess significant medicinal qualities that are particularly relevant to women's health concerns. They are also attributed with diuretic, demulcent, and diaphoretic properties (Duke and Ayensu, 1985). The rhizomes, in particular, stand out as a rich source of antioxidants, showcasing the plant's potential for various biological activities. While oxidation is a fundamental process for biological energy production in many organisms, the overproduction of reactive oxygen species during oxidative reactions is implicated in critical diseases (Mishra et al., 2011). *Canna edulis*, or Canna for short, emerges as an exceptional complementary starch in culinary applications, offering valuable dietary benefits. Antioxidants play a pivotal role in quenching oxidative chain reactions by neutralizing free radical intermediates through self-oxidation. Maintaining a proper balance in these networks within the human body is crucial, as imbalances have been linked to conditions such as cancer, atherosclerosis, diabetes, and premature aging. Numerous plants have been recognized as primary sources of antioxidant compounds (Larson, 1988; Thomas, 2009). These plants often contain phenolic compounds and flavonoids, natural antioxidants known for their anti-carcinogenic and anti-inflammatory properties (Mukhtar et al., 1994). While the phenol and flavonoid content of *Canna indica* has been documented, reports on the presence of antioxidants in this plant within the sub-Himalayan, West Bengal, and Darjeeling hill regions remain absent from the current literature (Atrooz et al., 2007: Vankar & Srivastava, 2008; Joshi et al., 2009).

Around the world, drug-resistant microorganism's accounts for at least 60 percent of hospital acquired infections (Edmond et al., 1999). Treatment involved is quite expensive and can result in longer hospital stays due to side effects of the drug being used and ultimately leading to higher healthcare costs (Webb et al., 2005). Another possible reason for still high mortality is difficult in treatment of intracellular infections. The efflux mechanisms of bacterial defence makes the removal and treatment of infectious disease difficult (Baker et al., 1995). Antimicrobial toxicity to healthy tissues cannot be ruled out posing significant limitation to their use (Mandell et al., 2001). On account of these issues, innovative approaches and novel antimicrobial from natural as well as chemical substances are required to make new army of drugs to control microbial infections.

Nanotechnology is highly interdisciplinary field with nanoparticles having novel use in medical science with great potential in therapeutic and diagnostic testing (Tartaj et al., 2003). The small size and large surface area of the nano particles, increases their interaction and facilitates various antimicrobial activities. Enhanced surface area leaves to increased surface energy, resulting in improved biological effectiveness (Reddy et al., 2007). Presently potential antimicrobial activity of metallic nanoparticles is being thoroughly explored and investigated.

2. Materials and methods

2.1 Collection of Sample

Fresh leaves and flowers of *Canna edulis* were collected from Kotlanala (Solan) and were transported to SILB Institute's microbiology lab where a further examination was done.

2.2 Preparation of extracts

Plant flowers and leaves were washed thoroughly with distilled water. The flowers and leaves were dried under sunlight for about 6-7 days. The dried Flowers and leaves of *C. edulis* were then powdered using a pestle and mortar, and then 10g of the powdered samples were taken and homogenized with 100 ml of ethanol. The supernatant containing the plant extract of each part was then transferred to a measuring cylinder and after the solvent evaporated, powder residue collected and weighed before used for bioassay (Alghamdi and Basher, 2020).

2.3 Synthesis of Ag nanoparticles

Aqueous extract of C. edulis was prepared using 10 g of powder of flower extract in 100ml of distilled water. The extract was placed in water bath at 60℃ for 30min. The extract was filtered through Whatman filter paper and used for further experiments. 2mM and 4mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. Now 10 ml of extract was added to 90ml of 2mM, 4mM of AgNO3 and was mixed well. It was then exposed to sunlight, until the colour of the solution becomes dark. It was then incubated in dark (to minimize the photoactivation of silver nitrate), at 37 °C under static condition for 72hours. After that, the extract was centrifuged at 7000rpm for 20 min, then the pellet and supernatant was collected in different containers. The pellet was washed with distilled water for two times and then collected by using ethanol and kept in oven for drying for 24hour into petri plates and supernatant was used for confirmation in synthesis of nanoparticles by taking OD in UV – Spectrophotometer (Aritonang et al., 2019).

2.4 Preparation of inoculum

Preparation of the bacteria stock was done to reproduce and rejuvenate bacteria. This was done by taking a bacterial colony with the help of inoculation loop into 5ml of nutrient broth and then incubated in orbital shaker.

2.5 Phytochemical analysis of nanoparticles

The extract was tested for the presence of bioactive compounds like alkaloids, phenolic compounds, carbohydrates, flavonoids and tannins by using the standard methods (Kim and Martin, 2006; Kim et al., 2016).

2.6 Characterization of Silver nanoparticles

To determine the optical properties of silver nanoparticles, UV-VIS analysis was done with the help of UV-VIS spectrophotometer. About 5ml of the sample was taken from stock and subjected to analysis with water as a blank reference. This method was carried out to confirm the synthesis of silver nanoparticles. UV-VIS spectra were observed in the range of 200-800nm (Phanjom et al., 2012).

2.7 Antimicrobial Activity

The antimicrobial activity of the plant extract was assessed using the agar well diffusion method. To examine this activity, Mueller-Hinton agar (MHA) medium was employed. The MHA medium was sterilized through autoclaving and subsequently poured into sterile petri plates under laminar air flow conditions. To initiate the process, a sterile cotton swab was immersed in a microbial suspension and then employed to inoculate the test plates. Both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Salmonella* spp.) were chosen as the target microorganisms for evaluating the antimicrobial effects. These microorganisms were sourced from the Microbiology Laboratory of Shoolini Institute and Business Management. Excess inoculum was eliminated from the swabs by firmly pressing and rotating them against the walls of the test tubes. The uniform distribution of inoculum on the surface of the MHA plates was achieved by repeatedly swabbing the plates with the cotton swab, including the rim of the agar. After a brief incubation period of 3-5 minutes, four wells were meticulously created in the MHA plates using a sterile corn borer. These wells were subsequently filled with extracts obtained from the leaves and petals of the plant. As a reference, antibiotic discs (Imipenem) were utilized as positive controls for the test organisms, while distilled water served as the negative control. Subsequently, the plates were incubated at a temperature of 37°C for a duration of 24 hours. Following the incubation period, zones of inhibition were visually identified, and the diameters of these inhibition zones were measured in millimeters. This methodology has been previously documented (Yadav et al., 2015; Pal et al., 2007; Bhuyar et al., 2021).

3. Results and discussion

3.1 Preparation of plant extract

The samples were collected from Kotlanala region of Solan city of Himachal Pradesh (30°54'24.5"N 77°06'36.3"E), India.

Figure 1 Preparation of flowers and leaves extract

The collected samples were transported to Microbiology laboratory of Shoolini Institute, Solan, India for further processing. The samples were converted into their respective powder form after washing and drying by using pestle –mortar as shown in Figure 1.

3.2 Preparation of nanoparticle extract

Nanoparticles from the leaf and flower extracts were prepared by following a standard protocol (Bhuyar et al., 2020) as given in Figure 2.

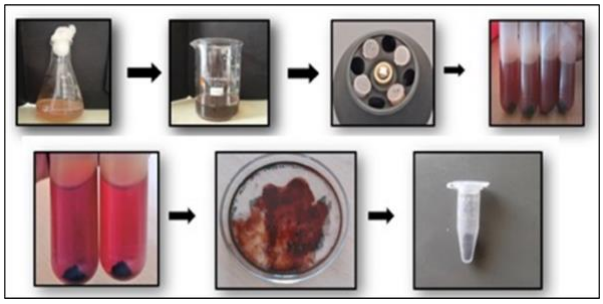

Figure 2. Extract preparation steps

3.3 Preparation of bacterial inoculum

Preparation of the bacterial culture was done to reproduce and rejuvenate bacteria. Four gram negative (*E. coli*, *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp.) and one gram positive (*Streptococcus* spp.) bacteria were used for antimicrobial assay. They were sub cultured and maintained in nutrient broth and nutrient agar as shown in Figure 3.

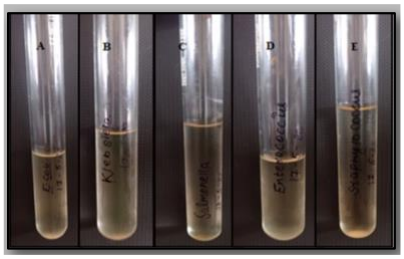

Figure 3. Inoculum preparations for antimicrobial activities (A) *E. coli* (B) *Klebsiella spp*. (C) *Salmonella spp.* (D) *Pseudomonas spp.* (E) *Staphylococcus aureus*

3.4 Qualitative analysis of phytochemicals

Results of phytochemical screening presented in Table 1 and 2, and the results of visual examination are presented in the Figure 4 and 5, reveals moderate concentration of alkaloids, carbohydrates, phenolics, flavonoids, tannin, and reducing sugars.

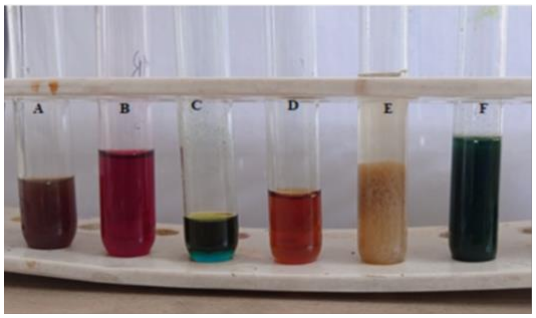

Figure 4. Qualitative phytochemical analysis of leaf extracts of *Canna edulis* (A) Alkaloids (B) Carbohydrates (C) Reducing sugar (D) Flavonoids (E) Phenolic compounds (F) Tannins

Table 1. Qualitative phytochemical analysis of leaf extracts of *Canna edulis*

S. NO.	Constituents	Test	Presence
1	Alkaloids	Dragendroff's test	++
2	Carbohydrates	Seliwanoff's test	+
3.	Reducing sugars	Fehling's test	+
4.	Flavonoids	Conc. H2SO4	+++
5.	Phenolic compounds	Lead acetate test	+++
6	Tannins	10% NaOH test	++

+++: Strongly positive, ++: Moderately positive, +: Weakly positive, -: Negative.

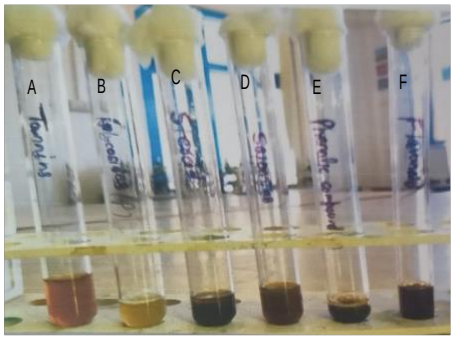

Figure 5. Qualitative phytochemical analysis of petal extracts of *Canna edulis* (A) Tannins(B) Alkaloids(C) Steroids (D) Phenolic compounds (E)Flavonoids

Table 2. Qualitative phytochemical analysis of petal extracts of *Canna edulis*

S. NO.	Constituents	Test	Presence
1	Alkaloids	Dragendroff's test	+++
2	Phenol	Iodine test	++
3.	Reducing sugars	Fehling's test	+
4.	Flavonoids	Conc. H2SO4	++
5.	Phenolic compounds	Lead acetate test	++
6	Tannins	10% NaOH test	+

+++: Strongly positive, ++: Moderately positive, +: Weakly positive, -: Negative.

In the results of phytochemical analysis of leaf extract of *Canna edulis*, the flavonoids and phenolic compound showed strongly positive presence, tannins and alkaloids showed moderate presence and reducing sugars and carbohydrates showed weak presence, while the results of phytochemical analysis of petal extract of *Canna edulis*, the alkaloids showed strongly positive presence, phenols, flavonoids and phenolic compound showed moderate positive presence and reducing sugar and tannins showed weakly positive results. Phytochemicals like carbohydrates, alkaloids, phytosterols, flavoniods, glycosides, fixed oil and phenolic compounds, fats, saponins and proteins of Canna indica L. were analyzed and claimed (Jeyaraman et al., 2011).

3.5 Characterization of Silver Nanoparticles

Synthesized silver nanoparticles were confirmed by using UV-spectrophotometer by placing supernatant in cuvette and taking OD at different wavelength. The production of Ag nanoparticles was monitored by UV - Vis spectra, with the maximum reduction and formation of metallic silver nanoparticles determined by the absorbance intensity. The intense peak at 400 nm by UV - visible absorption spectra confirmed the formation of colloidal Ag nanoparticles (Figure 6).

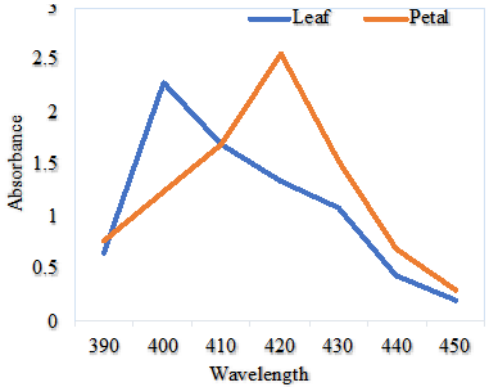

Figure 6. UV patterns of nanoparticles synthesized by the leaf extracts.

Table 3. Peak wavelength an absorbance of Ag nanoparticles in the aqueous extracts of fresh leaves and petals of *Canna edulis*

S No.	Wavelength (nm)	Leaf	Petal
1.	390	0.655	0.765
2.	400	2.28	1.243
3.	410	1.698	1.689
4.	420	1.343	2.56
5.	430	1.079	1.54
6.	440	0.448	0.698
7.	450	0.203	0.303

The characterization of silver nanoparticles derived from Canna edulis involved utilizing a UV-spectrophotometer to establish an optimal density curve for the plant's leaves and petals extracts. The UV-visible absorption spectra revealed distinctive peaks, with the leaf extract exhibiting a prominent peak at 400 nm, confirming the formation of colloidal silver nanoparticles.

Similarly, the petals extract displayed an intense peak at 420 nm in the UV-visible absorption spectra, further confirming the presence of colloidal silver nanoparticles. This phenomenon aligns with the findings of Patil et al. (2014), who utilized UV-visible (UV-Vis) spectroscopy to validate the biosynthesis of AgNPs (Otari et al., 2014). Furthermore, AgNPs were observed to exhibit a characteristic peak within the range of 400 to 500 nm, consistent with the study by Otari et al. in 2017. The presence of spherical nanoparticles was substantiated by the detection of a surface plasmon resonance (SPR) peak at 410 nm through UV-Vis spectrophotometry.

3.6 Antimicrobial study of Ag Nanoparticles against microorganisms

In the contemporary era, a concerning escalation in antibiotic-resistant bacteria has emerged as a significant challenge in effectively treating diverse microbial infections. Recognizing this predicament, the current study was initiated to synthesize nanoparticles with inherent antimicrobial properties using the aqueous extract of Canna edulis. The nanoparticles synthesized through the utilization of plant extract exhibited notable antimicrobial efficacy against a range of bacteria, encompassing both Gram-positive (*Staphylococcus aureus*) and Gram-negative species (*E. coli*, *Klebsiella* spp., *Salmonella* spp., and *Pseudomonas* spp.).

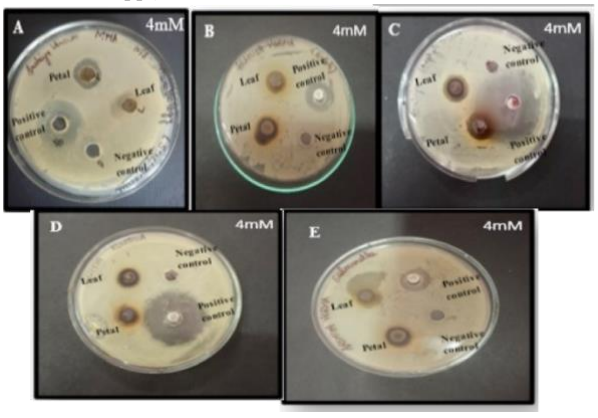

Figure 7. Antimicrobial activities of different extracts of *Canna edulis* with nanoparticles against gram-positive bacteria (A) *Staphylococcus aureus* (B) *E. coli* (C) *Pseudomonas* spp. (D) *Klebsiella spp.* (E) *Salmonella* spp.

A comparative assessment between the zone of inhibition observed for these nanoparticles and that of the standard Imipenem drugs was conducted, illustrating their potent antimicrobial potential. Employing the well diffusion method, the antimicrobial effectiveness of the synthesized nanoparticles was systematically evaluated. A graphical representation of the antimicrobial activity of the AgNPs is presented in Figure 7. This research underscores the promising role of Canna edulis-derived nanoparticles in combatting antibiotic-resistant bacterial strains.

The findings revealed that the leaf extract of *Canna edulis* exhibited superior effectiveness in comparison to the petal extract. This was evident from the larger zone of inhibition observed in the leaf extract as compared to the petal extract. However, a study conducted by Otari et al. (2017) on CELE (*Canna edulis*) indicated mild or no discernible antimicrobial activity against the tested human pathogenic microorganisms (Morones et al., 2005). In contrast, CELE-AgNPs demonstrated robust antimicrobial activity against a diverse array of microorganisms, including both gram-negative and gram-positive bacteria, as well as various fungal species. Notably, the antimicrobial potency of CELE-AgNPs surpassed that of the aqueous $AgNO_3$. Noteworthy differences in antimicrobial resistance were observed, with gram-negative bacteria displaying relatively higher resistance to the nanoparticles, potentially due to disparities in cell wall structure (Otari et al., 2017). Several bacterial strains were investigated for their susceptibility to the antibacterial effects of *Canna indica* rhizome essential oil. The oil exhibited moderate activity against Gram-positive bacteria like Staphylococcus aureus, low activity against gram-positive Bacillus subtilis, and no discernible activity against gram-negative *Escherichia coli* (Indrayan et al., 2011). Interestingly, fungal species demonstrated resistance to aqueous $AgNO_3$ while displaying susceptibility to CELE-AgNPs, signifying the potential of these prepared nanoparticles as antimicrobial agents. However, it is essential to assess the cytotoxicity of these particles against mammalian cells before considering their application in biomedical or industrial domains. Despite the promising antimicrobial properties demonstrated by the as-prepared AgNPs, their safety profile in relation to mammalian cells must be thoroughly examined.

Table 4. The Zone of inhibition (mm) obtained by the well diffusion method

Bacteria	Leaf of 2mM	Petal of 2mM	Leaf of 4mM	Petal of 4mM	Distilled water	Imipenem (5mg)
Salmonella spp.	No zone	No zone	17±1	15±3	No zone	28±3
Staphylococcus aureus	No zone	No zone	15±1	14±2	No zone	30±2
Klebsiella spp.	No zone	No zone	17±1	14±2	No zone	27±3
E. coli	No zone	No zone	18±2	12±2	No zone	28±2
Pseudomonas spp.	No zone	No zone	16±1	15±3	No zone	31±1

± sign for standard deviation, experiment was performed in triplet

4. Conclusion

This study provides a novel, green route for synthesizing silver nanoparticles using *Canna edulis* extract. *Canna edulis*, as complementary starch is good food material. Various research has been conducted to evaluate the traditional uses of *Canna edulis* and all the research supported the traditional claims. In the present study, nanoparticles were prepared from the extract of leaf and petals. The results showed that the leaf extracts of *Canna edulis* given greater zone of inhibition against the bacteria than the petal extract. The characterization of nanoparticles of the extract showed the optimum density from which the maximum peak was obtained at 400nm for leaf and 420nm for petal respectively. The phytochemical analysis of leaf extract of *Canna edulis* concluded that alkaloids showed strongly positive results and in petal extract, flavonoids and phenolic compounds gave strongly positive results. The resulting AgNPs, enriched with phytochemicals, exhibit strong antimicrobial properties, highlighting their potential for therapeutic and environmental applications.

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