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GREEN SYNTHESIS AND STUDY ON ANTIMICROBIAL ACTIVITY OF NANOPARTICLES FROM FLORAL EXTRACT OF *CHRYSANTHEMUM INDICUM*

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ABSTRACT

Chrysanthemum indicum widely available flowers have long been used in traditional Korean and Chinese medicine to treat inflammatory diseases. C. indicum is well known for its use in food additives for enhancing flavors, in teas and alcoholic beverages from the ancient times. Now a day's C. indicum has been used as an herbal medicine, which is prescribed for anti-inflammatory, analgesic, antipyretic purposes and the treatment of eye diseases. The bioactive components in C. indicum are glycosides and flavonoids has the ability to act as antibiotic against many species of disease causing bacteria. However, the pharmacological activity and bioactive constituents of this natural medicine are left uncharacterized. C. indicum can be used in the form of ethanolic extract, essential oil and air dried and processed flowers. The present study reveals the medicinal use and antibacterial properties of ethanolic extract of Chrysanthemum indicum and its nanoparticles against oral, skin, enteric and Nosocomial bacteria. It shows effectivity against oral, skin and enteric bacteria while nosocomial bacteria shows resistance.

KEY WORDS

antibacterial properties, Chrysanthemum indicum, glycosides and flavonoids, herbal medicine, Silver nanoparticles

INTRODUCTION

Chrysanthemum indicum is an erect, aromatic, perennial plant, which is harvested from the wild for local use as a food and medicine. It is a plant of temperate origin; it can be grown successfully in tropical areas. The phytoconstituents isolated from plants have chemical substances which bind to effective target sites in human body. The most important bioactive compounds from plants are alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins, sugars, cumarins, proteins, and phenols [1]. Flavonoids are proved to be important bioactive in herbal plants Several flavonoids and glycosides have been isolated from medicinal plants and which play a vital role in healthcare systems of the most traditional and modern medicines. *Chrysanthemum indicum* has been used as a herbal medicine, which is prescribed for anti-inflammatory, analgesic, antipyretic purposes and the treatment of eye and skin diseases. The *C. indicum* has a long history using as an Oriental traditional medicine for the treatment of several infectious diseases such as pneumonia, colitis, stomatitis, cancer, fever, and used to treat migraine, pertussis, and hypertensive symptoms. The phytochemicals of *C.indicum* leaves are also responsible for anti-proliferative effect in yeast can be purified and explored for anticancer potential in humans. [2]

Edible parts of Chrysanthemum indicum:

The flower heads are pickled in vinegar. Young leaves - cooked. An aromatic tea is made from the leaves. [3]

Chrysanthemum Tea: The tea is a natural coolant; tasty beverage especially benefits the upper respiratory tissues. It lowers the fever and relieves headache. It is a good source of beta – carotene. It also addresses a number of skin disorders and helps to boost immune system. [4]

Importance of Green synthesis

The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Plant-mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants. Novel methods of ideally synthesizing NPs are thus thought that are formed at ambient temperatures, neutral pH, low costs and environmentally friendly fashion. Plants are nature's "chemical factories". They are cost efficient and require low maintenance. [5]

The different parts of plant extracts are ecofriendly, economical and safe for the synthesis of nanoparticles. Use of flower extract for synthesis of nanoparticles has an added advantage of environmental friendly. Flowers are normally thrown away into the environment, so evaluating therapeutic value of discarded material is a novel idea. [6] Synthesis of nanoparticles is presently an important area of research, searching for an eco-friendly manner and green materials for current science. A number of AgNP have been developed by a chemical approach, a physical approach, and a recently developed biological method. [7]

The aim of the study was green synthesis of silver nanoparticles and to study the antibacterial activity against oral, skin, enteric and Nosocomial bacteria.

MATERIAL AND METHODS

Flower collection:

C. indicum flower were collected from local nurseries in Ambarnath city. Flowers with yellow petals were picked by hands (Fig. 1). After full blooming and spread under sun light for 3 days then they were moved to shadow until they are completely dry, and it can be known through smashing of their petals.

Figure 1: *Chrysanthemum indicum* Flower

Extraction:

Plant tissue homogenization was performed by using Methanol as a Solvent. After 24 hours, the extract was filtered, and it was kept in the hot air oven for 3-4 hours for solvent evaporation. The dried extract precipitate was scrapped into the powder form and diluted as per the requirements in phosphate buffer. [8]

Green synthesis of silver nanoparticles:

Aqueous extract was prepared by dissolving 5 g of powder in a 150-ml flask with 50 ml of sterile distilled water and boiled for 5 min and then filtered with Whatman filter paper no. 1 and stored at 4 °C for further experiments. The filtrate (2 ml) was treated with 1-mM AgNO3 solution (150 ml) in an Erlenmeyer flask at room temperature, resulting to the conversion to reddish brown color indicating the formation of silver nanoparticles (AgNPs) within 5 min. [9]

Characterization of AgNP's:

The synthesized AgNPs were characterized by UV–vis spectrometer in the wavelength of 350– 800 nm. [10]

Antimicrobial activity:

The antimicrobial activity of both the methanolic extract and AgNp's of *C. indicum* flower evaluated against both Gram-positive bacteria (*Staphylococcus aureus, Bacillus Subtilis*), Gram negative bacteria (*Escherichia coli, Pseudomonas aerugenosa, Salmonella paratyphi B*) and fungi (*Aspergillus niger, Mucor spp*.). In agar diffusion method, 24 hrs old cultures were used to study

antimicrobial activity. Inoculums were inoculated into sterile molten Muller Hinton agar and poured into petridishes. Plates were solidified and surface dried before inoculation. Wells were prepared with the help of cork borer and filled with diluted concentrations (5µg/ml-20µg/ml) of flower extract. Then petridishes were incubated at 37 °C for 48 hrs. After incubation zone of inhibitions were recorded in mm. [11]

RESULT AND DISCUSSION

Methanolic extracts were prepared by using dried flowers (Fig.2). After evaporation aqueous extracts were prepared with different dilutions 0f 5µg/ml-20µg/ml (Fig.3).

Methanolic Extract

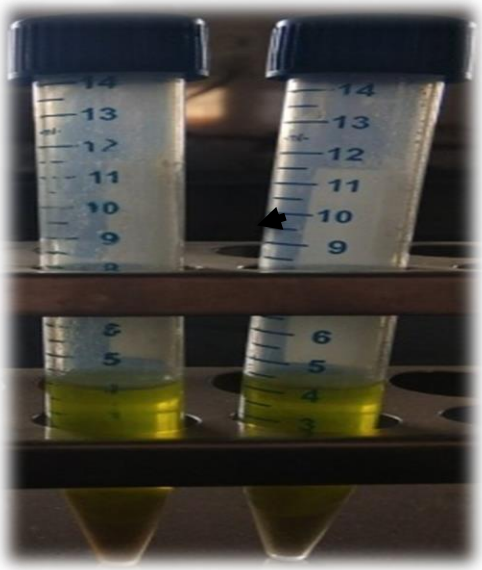


Figure 2: Methanolic extract of *C.indicum* flower.

Aqueous extract

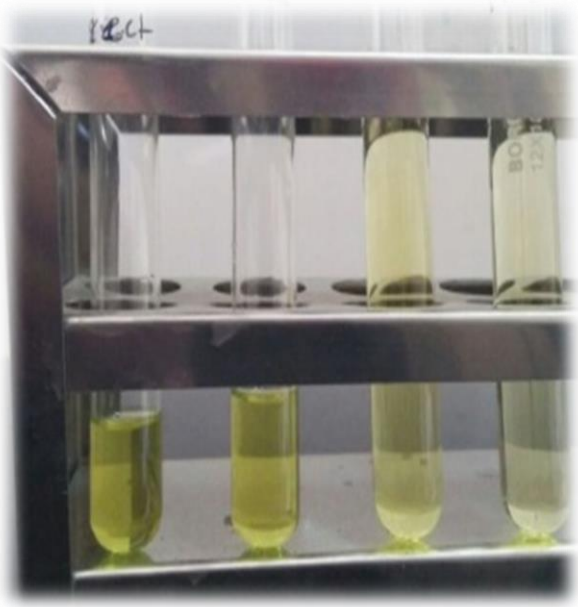
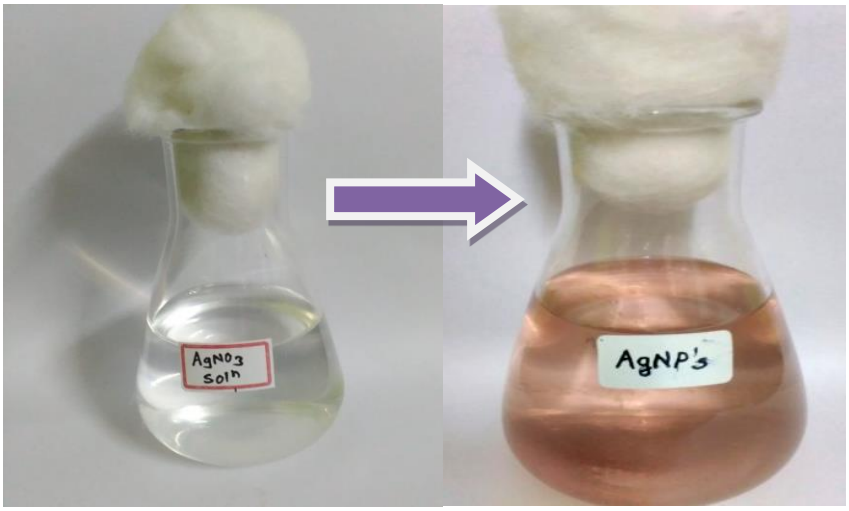


Figure 3: Aqueous extract of *C.indicum* flower.

Synthesis of Silver nanoparticles.



(A) **(B)**

Fig. 4: Green synthesis of silver nanoparticles from *C. indicum* flower extract
(A): 1mm $AgNO_3$ + Flower extract; (B): Formation of silver nanoparticles after 5 min.

In this study, Ag NPs were synthesized by green method using an aqueous floral extracts of *C. indicum* (Fig.4). The change in colorless silver nitrate solution into reddish brown indicated that the reaction was due to the bio reduction of silver. It was reported that the Ag NPs synthesized using Datura petal containing alkaloids, proteins, enzymes, amino acids, alcoholic compound, and polysaccharides were responsible for the reduction of silver ions to nanoparticles. [12]

The formation of Ag NPs in low level concentration was confirmed by one of the imperative technique UV–Vis spectroscopy analysis. In this study, the absorption spectra were noted at 435 nm (Fig. 5), and a broadening of the peak indicated that the particles were polydispersed. A similar report was observed by Selvaraj *et al.*2014. [13]

It can be observed from table 1 and 2 that the highest antimicrobial activity was shown against Gram positive *S.aureus* (21mm) by silver nanoparticles than methanolic undiluted extract of *C.indicum* (18mm) (Fig. 6) .While silver nanoparticles shows highest antibacterial activity against Gram negative *E.coli* (18mm) than methanolic extract (16mm) (Fig. 7). *S.aureus* is more sensitive then *E.coli* against nanoparticles [14,15] .The lowest antimicrobial activity was shown against *A.niger* where *P.aeruginosa* shows resistance towards all the dilutions of extracts and nanoparticles.

SR.NO.	DILUTIONS USED	ZONE OF INHIBITION (mm)				
		S.aureus	***E.coli***	***P.aerugenosa***	***A.niger***	***C.albicans***
1.	5µg/ml	18	16	No zone of inhibition	14	15
2.	10µg/ml	11	15	No zone of inhibition	11	12
3.	15µg/ml	10	12	No zone of inhibition	8	10
4.	20µg/ml	10	10	No zone of inhibition	8	10

Table 1: Antimicrobial activity of methanolic extract.

SR.NO.	DILUTIONS USED	ZONE OF INHIBITION (mm)				
		S.aureus	***E.coli***	***P.aerugenosa***	***A.niger***	***C.albicans***
1.	5µg/ml	21	18	No zone of inhibition	16	18
2.	10µg/ml	18	15	No zone of inhibition	12	12
3.	15µg/ml	16	12	No zone of inhibition	8	10
4.	20µg/ml	11	10	No zone of inhibition	7	8

Table 2: Antimicrobial activity of biologically synthesized AgNP's.

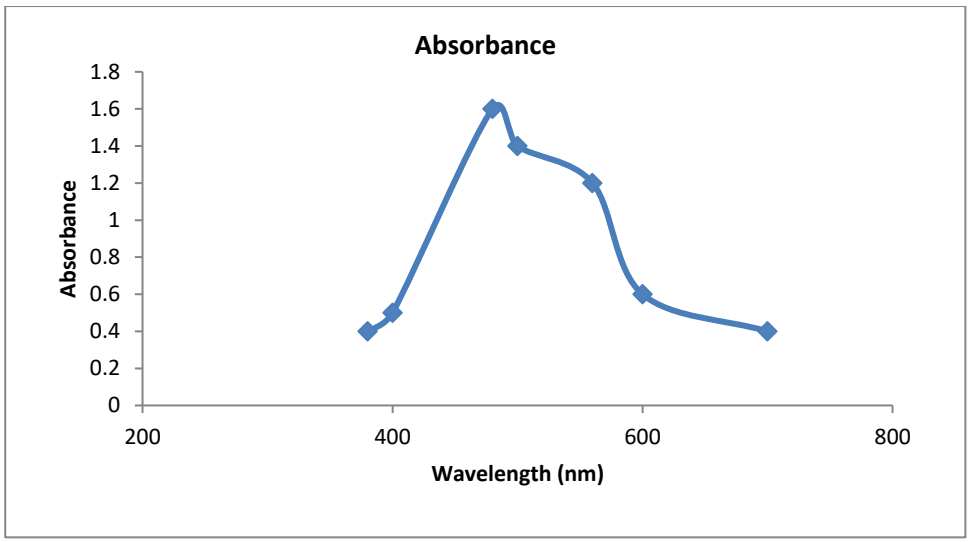


Figure 5: UV absorption spectra of synthesized nanoparticles.

Study of antibacterial activity

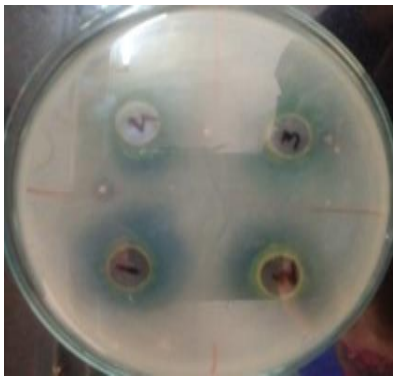


Figure 6: Antibacterial activity of Silver Nanoparticles on Gram positive *S. aureus*

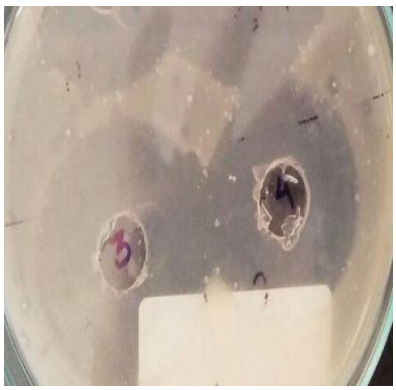


Figure 7: Antibacterial activity of Silver nanoparticles on Gram negative *E. coli*.

CONCLUSION

AgNPs were successfully synthesized using floral extract of *C. indicum*. The results recorded from UV–visible spectrum support the biosynthesis of Ag NPs., Highest antimicrobial activity was shown against Gram positive *S.aureus* (21mm) by methanolic undiluted extract of *C.indicum* in comparison with Gram negative *E.coli* (18mm). The lowest antimicrobial activity was shown by *A.niger* where *P. aeruginosa* shows resistance toward all the dilutions of extract. Maximum antimicrobial activity shown against skin infection causing organism. Hence, it is therefore suggested that extract can be used for ointment preparations for skin diseases.

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