Evaluation of Antimicrobial Potential of a Gel loaded with Silver Nanoparticles of *Argemone mexicana* for Topical Application

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Abstract: *Argemone mexicana* is widely known for its antibacterial potential in the traditional system of medicine. With this background, the present study was designed to formulate a gel incorporated with nanoparticles synthesized from *Argemon mexixana* for topical application. Green synthesis of nanoparticles was carried out from the whole plant extract and latex of *A. mexicana*. The biosynthesized nanoparticles were characterized by SEM-EDAX, HR-TEM, SAED, XRD before it was incorporated into the gel. The biosynthesized nanoparticles were found to be stable, uniform, spherical, and ranged from 9.21nm to 14.03nm in size. The SAED pattern reveals the presence of a varying degree of crystallinity of nanoparticles. The physicochemical parameters of the gel such as pH, homogeneity, grittiness, viscosity, spreadability, and extrudability were characterized. Both the formulation showed the nanoparticles are well dispersed in the gel with pH (6.90, 6.92), viscosity (1542 scps, 1583 scps), spreadability (5.7 cm/sec, 5.90.1 cm/sec), and extrudability of 86% and 89% respectively. The gel loaded with nanoparticles exhibited strong antimicrobial potential against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* as compared to the crude form of the plant extract and latex. Among the two formulations, the highest zone of inhibition (22mm, 16mm, 13mm) was observed by the gel incorporated with methanol extract nanoparticles. Our result demonstrates the nanoparticulate gel of *A. mexicana* can be effectively used as topical gel for gram positive and negative bacterial infection.

Keywords: *Argemone mexicana,* Topical Application, Herbal Gel, Silver Nanoparticles

1. INTRODUCTION

Argemone mexicana Linn. popularly known as Prickly poppy is commonly distributed across India and also considered as a medicinal weed. This plant is found in barren lands, cultivating fields, newly excavated sites, and by the side of roads and rivers (Fig. 1). The stem of the plant is spiny, leaves are irregular in shape, and produces deep yellow color latex. *A. mexicana* is known for its multifold medicinal uses in the traditional medicine.

Fig. 1 *Argemone mexicana* growing in natural environment

A. mexicana have been used against skin infection, worms, nail infection, ulcers, and many more. Seeds and seed oils are used as a laxative, normalizing blood circulation, curing dysentery, headache, reducing cholesterol level, itchy skin and also used in woods to protect against termites (Chopra *et al.,* 1956; Bose *et al.,* 1963; Ambasta, 1986;

Manandhar *et al,* 2002: Prajapati *et al.,* 2003; Savithramma *et al.,* 2007; Makhija & Khamar, 2010; Minu *et al.,* 2012). The plant flowers profusely throughout the year, which is used for the treatment of cough, relieving chest congestion, asthma, and hypertension (Chevallier A, 1996; Manandhar *et al.,* 2002; Brahmachari *et al.,* 2010; Bieski *et al.,* 2012). The yellow latex of the plant is reported to be useful in many ailments like skin diseases, nail infection, jaundice, blisters, conjunctivitis, leprosy, inflammations, and burning sensation (Sharannappa and Vidyasagar, 2014). The latex of *A. mexicana* is used to get relief from rheumatic pain and to reduce the eye infection. Several researchers also reported its positive effect on wound healing, larvicidal properties, hypoglycemic activities, antimicrobial activities, anticancer activities, anti-HIV properties, and hepatoprotective activity (Izzo *et al.,* 1995, Dash *et al.,* 2011, Shaukat *et al.,* 2002, Adolfo and Michael , 2005, Siddiqui *et al.,* 2002, Osho and adetunji, 2010). Studies also reported the presence of various biologically active phytoconstituents in *A. mexicana* with potential therapeutic attributes. Chemical constituents investigation confirms the detection of alkaloids like isocorydine, allocryptopine, cheilanthifoline, scoulerine, berberine, protopine, steroids like beta amyrin, flavonoids like cysteine, phenylalanine, phenolics like 5,7-Dihydroxy chromone-7-neohesperidoside, ferulic acid, benzoic acid , caffeic acid, cinnamic acid, tannic acid (Israilov and Yuhusov, 1986; Chang *et al*., 2003; Haisova *et al*., 19; Fletcher *et al*, 1993; Tripathi *et al*, 1999; Sukumar *et al*., 1984; Bhardwaj *et al.,* 2012). Plant-based nanoparticles are gaining immense importance due to their heightened biological activity even with a negligible amount. Among various approaches used for nanoparticle synthesis, the green synthesis method became more popular as it is safer, eco-friendly, less toxic, and less hazardous than other methods. The green synthesis method is also cheaper, convenient and does not require energy, temperature, or toxic chemicals (Sintubin, 2011; Kumar 2014). Among various metals used for nanoparticle synthesis, silver nanoparticle is of great choice because of its diverse activity especially antimicrobial and catalytic activity (Mashwani, 2015; Rostami, 2016). Strong antibacterial activity of crude extract of *A. mexicana* against a range of microorganism and contagious pathogens that causes skin infection has been reported (Rahman *et al*., 2009; Singh *et al*, 2009; Rosas-Pinon *et al*., 2012; Alagesaboopathi and Kalaiselvi, 2012, Doss *et al*., 2012; Jain *et al*., 2012; Pandey and Karanwal, 2011; Abubacker and Ramanathan, 2012). Due to its lesser cost and availability, our research is aimed to find the antibacterial potential by synthesizing nanoparticles from this plant. The active compounds of *A. mexicana* are incorporated into the gel in the form of nanoparticles which could prevent the skin infection more effectively.

2. MATERIAL AND METHODS

Collection of plant material

Whole plants of *Argemone mexicana* were collected from the surrounding places of the college campus of Padmashree Institute of Management and Sciences, Bangalore. Latex from young healthy plants was collected in a sterilized bottle with the help of a cotton wick. The plant materials were washed carefully with tap water to remove dirt and sun-dried for one week. The plant materials were powdered using a mechanical grinder and was kept in an airtight container for further use.

Solvent Extractions

The crude powder of *A. mexicana* (10gms) was mixed in 100 ml of methanol and ethyl acetate separately and boiled for one hour in a water bath at 5^0C below the boiling temperature of the solvent. The solution was filtered through Whatman's filter paper and the filtrate was concentrated in a vacuumed oven. The percentage yield of the extract was determined.

Synthesis of nanoparticles

The nanoparticles synthesis was carried out by silver nitrate reduction method using solvent extracts and latex of *A. mexicana*. The latex was diluted to 4% by adding distilled water and five ml of diluted sample was added to 45 ml of 1mM $AgNO_3$. The solution was mixed uniformly and kept on water bath at 80℃ for 2 hours. On the other hand, 5ml of methanol extract was also added to 45ml of 1mM $AgNO_3$. The visible change in color intensity of the solution was recorded at an interval of thirty minutes. The solutions were poured in petri plates and dried at 60℃ to obtain powder.

Characterization of nanoparticles

UV–Vis Spectroscopy:

The synthesized nanoparticles were analyzed in the instrumentation facility of sophisticated test and instrumentation center (SAIF, Kochi, India). The biosynthesized silver nanoparticles (AgNPs) solution was collected every 15 minutes intervals. The reduction of Ag+ and synthesis of silver nanoparticles were analyzed with Elico SL210 UV–Vis Spectrophotometer in 300–700 nm range. The maximum web length of absorption was determined.

Scanning Electron Microscopy (SEM)

The purity and morphology of the nanoparticles are characterized by using scanning electron microscope integrated with energy dispersive X-ray analyzer (SEM-EDAX). SEM analysis was performed using the JEOL 6390LA model instrument (Jeol, Japan). The sample was prepared by dropping a small amount on a carbon-coated copper grid and kept for drying using mercury lamp prior to scanning.

Transmission electron microscopy (TEM)

The size and shape of the synthesized nanoparticles were measured by high-resolution transmission electron microscopy (HR-TEM). The microscopy (TEM) was carried out using the JEOL JEM-2100 model instrument operated at an accelerated voltage of 200kV. The nature of the nanoparticles was analysed by the XRD pattern at 2Ɵ ranges from 0 to 100°. The XRD was carried out by casting the powdered silver nanoparticles on a glass slide and air-dried under ambient conditions. The data was recorded by copper K-α radiation with λ of 1.55A° at a voltage of 40 kV and a current of 40 mA with scanning interval at 10°/min.

Formulation of gel loaded with nanoparticles

The gel was prepared using the cold mechanical method. Appropriate quantity of carbapol- 934 (0.1%) was gently dispersed in 50 ml of double-distilled water and left overnight with constant stirring for complete dissolving the polymer. In a test tube, 0.5% methylparaben (0.2ml) and 0.2% propylparaben (0.1ml) were mixed in 5ml of distilled water separately by keeping on a water bath. The solution was cooled and 5% of glycerol (5ml) was added and mixed thoroughly. The synthesized nanoparticles were added subsequently to the prepared Carbopol-934 and solution of methylparaben and propylparaben. The pH was adjusted up to 7 by adding Triethanolamine (TEA). The volume was made up to 100ml by adding required amount of double distilled water and mixed by magnetic stirrer until the gel becomes smooth and uniform.

Physicochemical evaluation of gel

The physical parameters of the formulation such as color, consistency and texture were checked visually at regular interval.

Homogeneity

The gel loaded with nanoparticles was tested for homogeneity by visual inspection after the gel has been set in the container. The appearance of the gel and the presence of any aggregates were also verified.

Grittiness and pH

The gel formulation was observed under a microscope to detect any unwanted particles. The pH of the aqueous solution (1%) of the formulation was measured by a calibrated digital pH meter at a constant temperature.

Viscosity

The viscosity of the gel was determined using Brookfield digital Viscometer according to the instruction provided by the manufacturer. The gel was allowed to settle down at least one hour before the reading was taken.

Spreadability

The gel of three gm was placed on the standard glass slide (6.0 cm) and pressed to form uniform thickness with another slide. The gel on the outer surface of the slides was cleaned by gentle wiping. Slides are fixed in a wooden block so the upper slide will slip with 20 g of weight. The Time taken for the upper slide to move 6.0 cm was recorded. The experiment was repeated in triplicate and spreadability was calculated:

$$\text{Spreadability} = \frac{\text{Weight} \times \text{Length}}{\text{Time}}$$

S= Spreadability
m=Weight along with upper slide (20 g)
l= Glass length (6.0 cm)
t= Time taken (second)

Antimicrobial activity of gel loaded with nanoparticles

The gel loaded with nanoparticles was subjected to evaluation for antimicrobial activity against different gram-positive and negative bacterial strain (Kirby-Bauer Methods described by Drago *et al*., 1999). The microorganisms selected for this study are *Escherichia coli, Bacillus subtilis, Staphylococcus aureus*. Wells of pre-inoculated plates were filled with 50µl of each nano-gel sample (gel incorporated with nano-particles of methanol extract and latex). The plates were incubated at 37° C for 24 hours and gels were allowed to diffuse on a horizontal surface. The antibacterial activity in terms of the zone of inhibition was measured and expressed in millimeter.

3. RESULTS AND DISCUSSION

Nanoparticle synthesis

The percentage yield of the extract from 10 grams of dry plant material was estimated. The yield of 2% and 3.8% was observed when methanol and ethyl acetate was used as solvent. The UV spectroscopy method was used to confirm the formation of the nanoparticle. The change in color from light yellow to dark brown prooves the reduction of Ag^+ by the *A. mexicana* extract and latex. This change of color intensity is attributed to the surface Plasmon ratio of metallic nanoparticle exerted by their particle size, chemical neighborhood, and dimeric medium (Ibrahim, 2015; Dada *et al*., 2017). The absorption peak was observed at 450 nm after 40 minutes in methanol extract nanoparticles and 310 nm after 50 minutes in nanoparticles of latex respectively. No higher absorption peak was observed beyond this incubation time. The active ingredients present in the plant are also reported to contribute the nanoparticles synthesis (Das and Burmon, 2014). The stability of the nanoparticles was observed by keeping the synthesized nanoparticles up to 10 days under normal room temperature. Similar absorption values are also recorded after 10 days of incubation, which indicates the long stability of the nanoparticles under normal conditions.

Characterization of nanoparticles

The synthesized nanoparticles were characterized using scanning electron microscopy (SEM) integrated with SEM-EDAX). The microscopic image showed spherical shape of nanoparticles in both methanol extract and latex [Fig. 2 (a, b)]. The EDAX spectra also confirmed the presence of silver elements in both nanoparticles with the highest absorption peak at 3keV [Fig. 3 (a, b)].

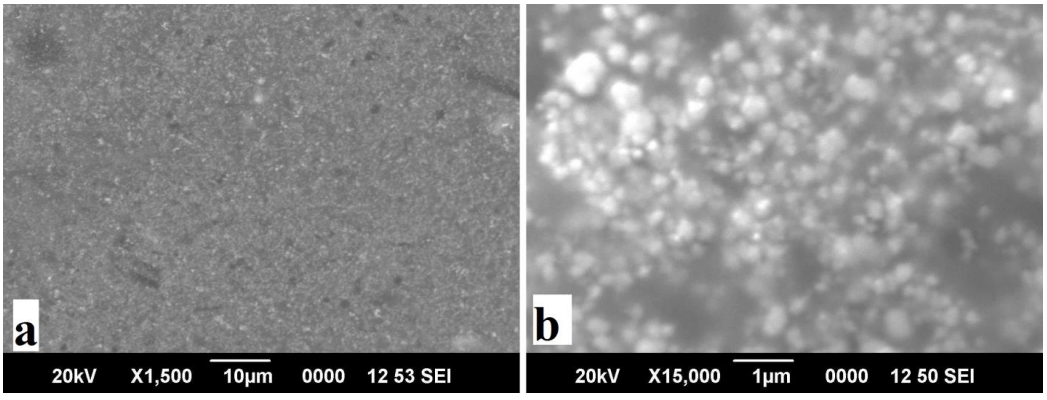

Fig. 2 Scanning electron microscopic (SEM) images of biosynthesized silver nanoparticles **A:** SEM image of methanol extract nanoparticle of *A. Mexicana* **B:** SEM image of silver nanoparticle of latex of *A. mexicana*

The optical peak of around 3keV is the typical characteristic feature of the metallic silver absorption peak due to its surface plasmon resonance (Kaviya *et al*., 2011; Markus *et al*., 2017; Naggar *et al*, 2017). An additional strong peak

for carbon was observed in both the nanoparticles of methanol extract and latex which may be attributed to the kind of grid used during analysis.

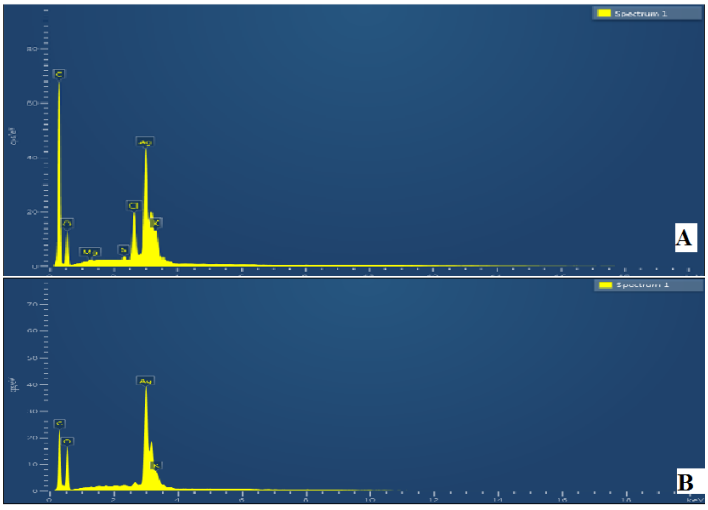

Fig. 3 The energy dispersive X-ray analysis spectrum of biosynthesized silver nanoparticles **A**: Spectrum (EDAX) of silver nanoparticles of latex of *A. mexicana* **B**: EDAX spectrum of silver nanoparticles of methanol extract of *A. mexicana*

The presence small peak of Cl, S, O, K and Mg peak is due to the phytochemicals in the extract attached to the surface and take part in the formation of the nanoparticle. This may be considered as an added advantage of green synthesis method of nanoparticles rather than using toxic chemicals.

The size and morphology of the nanoparticles play a crucial role in the therapeutic activity of the formulation. Therefore, to gain further insight into the size and morphological structure of the biosynthesized nanoparticles, HRTEM analysis was performed. The HRTEM image of the nanoparticle reveals the formation of uniform and spherical nanoparticles. The nanoparticles were observed to be well dispersed in the gel, ranging from 9.21nm to 14.03nm in size in case of nanoparticles prepared with latex and 11.22 nm to 27.98 nm in methanol extract nanoparticles [Fig. 4 (a, b)].

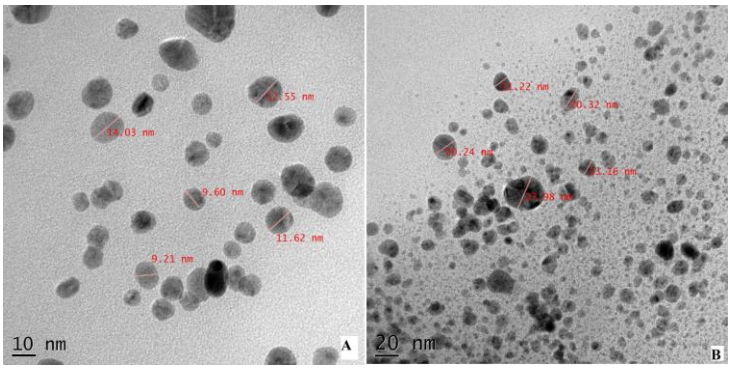

Fig. 4 Morphological characterization of silver nanoparticles (AgNPs) using high-resolution transmission electron microscopy **A:** HR-TEM images of silver nanoparticle of latex of *A. mexicana* **B:** HR-TEM image of silver nanoparticles of methanol extract of *A. mexicana*

The selected area electron diffraction (SAED) proved to be an important crystallographic technique where electrons are diffracted in a selected area that provides information about the crystalline or amorphous nature of the materials. To know the nature of the nanoparticles, selected area electron diffraction study was performed. The SAED graph of nanoparticle reveals the presence of a varying degree of crystallinity. The methanol extract shows strong and broad diffused rings with few discrete reflections which is the typical characteristic of polycrystalline material (Fig. 5). However, in the case of nanoparticles synthesized from latex the ring shows a well defined crystalline diffraction pattern of SAED (Fig. 6).

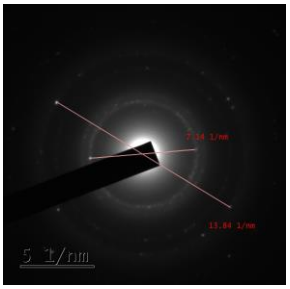

Fig. 5 Selected area electron diffraction image of synthesized silver nanoparticles of methanol extract of *A. mexicana*

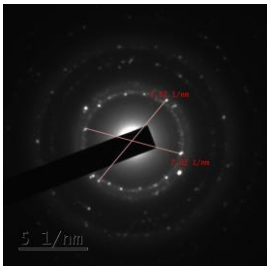

Fig. 6 Selected area electron diffraction image of synthesized silver nanoparticles of latex of *A. mexicana*

The XRD pattern of nanoparticles from latex showed diffraction peak at 2Ɵ are 38.12°, 35.46°, 29.60°, 24.27°, 21.66°, 19.59°, 32.74°, 27.70°, 26.73° (Fig. 7). Similarly, the XRD pattern of methanol extract nanoparticles assigned to diffraction peak at 2Ɵ is 32.41°, 38.31°, 46.39°, 27.99°, 34.00°, 44.37°, 54.94°, 57.60°, 64.58° respectively (Fig. 8).

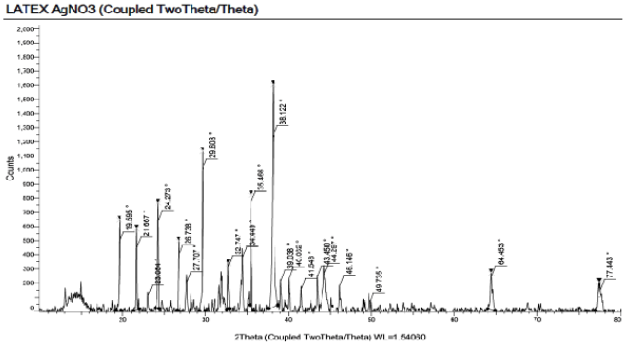

Fig. 7 X-ray diffraction (XRD) pattern of synthesized silver nanoparticles of latex of *A. mexicana*

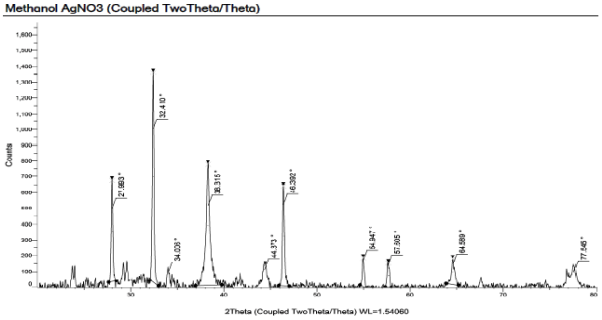

Fig. 8 X-ray diffraction analysis of synthesized silver nanoparticles of methanol extract of *A. mexicana*

The peak corresponds to the different planes due to the presence of a significant number of crystallites in each plane that are properly oriented to diffract. The XRD pattern where the number of crystallites presents in different possible plane reconfirms the polycrystalline nature of synthesized nanoparticles.

Physicochemical Characteristic of nanoparticulate gel

Physicochemical parameters such as homogeneity of color, presence of any foreign particles, ease of removing the gel, pH, and viscosity of the gel are evaluated. The prepared gel was slightly yellowish in color, smooth, homogeneous, free from lumps and foreign particles as observed under a light microscope [Fig. 9 (a, b)]. The formulation can be washable very easily, pH was found to be 6.90 and 6.92 in both the gel, viscosity (1542 scps., 1583scps.), spreadability (5.7 cm/sec, 5.90.1 cm/sec), and extrudability was 86% and 89% respectively. Gels incorporated with herbal nanoparticles are getting much popular due to their ease of application, less toxicity, and efficiency in drug delivery.

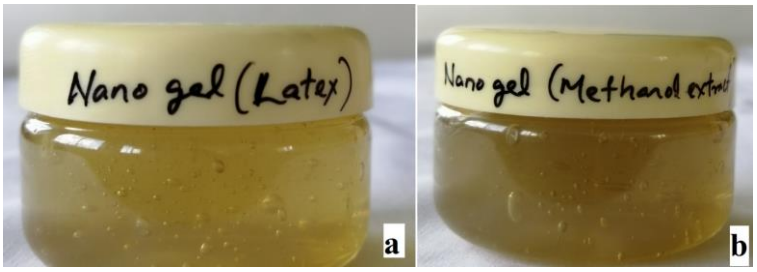

Fig. 9 Gel formulation loaded with silver nanoparticles **A:** Gel loaded with silver nanoparticles of latex of *A. mexicana* **B:** Gel loaded with silver nanoparticles of methanol extract of *A. Mexicana.*

The plant extract and latex are rich in bioactive phytoconstituents which are highly efficient in its nanoform when introduced into the gel for topical application. A suitable protocol was established for the formulation of gel loaded with well-dispersed nanoparticles. The stability of the formulation was studied up to one month at different temperature conditions and it was noted that the gel was quite stable up to 48°C. However, beyond 48°C discoloration of the gel and water exudates in the gel was observed. This may be due to the weakening of the intermolecular forces of interaction and reduction in the polymerization strength of the gel (Carter, 2000). The above characteristic of the gel suggests its suitability for topical application.

Antibacterial activity of the formulation

The gel incorporated with biosynthesized nanoparticles of methanol extract and latex was screened for their antimicrobial potential by assessing zone of inhibition diameter. Both the formulated nano gel exhibited significant antimicrobial activity against tested bacteria (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis*). The bactericidal activity indicates that gel loaded with nanoparticles is comparatively much efficient than its crude form of the plant extract and latex (Table No.1).

Table 1: Anti-microbial assay of the gel loaded with nanoparticles, crude extract and latex

SL. No	Test Organism	Zone of inhibition (mm) Gel incorporated with nanoparticles (methanol extract)	Zone of inhibition (mm) Gel incorporated with nanoparticles (latex)		Zone of inhibition (mm)	
				Methanol extract	Latex	Ethyl acetate extract
1	*Escherichia coli*	*22*	*18*	*14*	*11*	*8*
2	*Bacillus subtilis*	*16*	*13*	*8*	*7*	*4*
3	*Staphylococcus aureus*	*13*	*10*	*6*	*4*	*3*

The highest zone of inhibition of 22 mm was observed when gel was loaded with nanoparticles synthesized from methanol extract tested against *E. coli*. Among the two nanoparticulate gel, the gel loaded with nanoparticles of latex was found to be comparatively less effective. However, both the gel produced significant antibacterial potential (Fig. 10). The least zone of inhibition diameter of 3mm was observed in the case of ethyl extract crude extract against *Staphylococcus aureus*. As the ethyl extract was least effective against all the bacteria tested, it was not considered for nanoparticle synthesis. Latex showed the highest activity with 11mm zone of inhibition against *E. coli*. The strong antibacterial activity suggests, the nanoparticulate gel could be used for antibacterial topical application. The

mechanism by which the silver nanoparticles exert its antibacterial activity is probably by attaching to negatively charged cell wall and successively disrupting the bacterial cell. Several studies also demonstrated the silver ion causes toxicity to the cell by inhibiting the functioning of several cellular enzymes and ATP synthesis (Chairuangkitti *et al*., 2013, Grzelak *et al*., 2018).

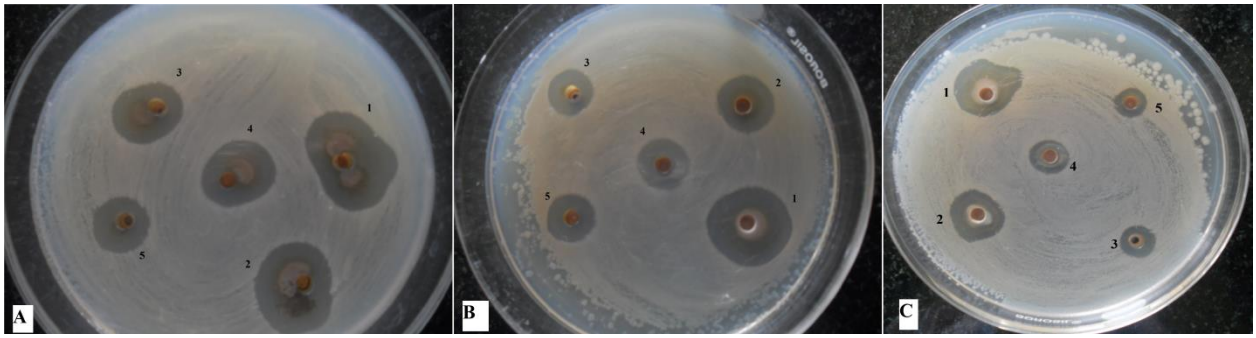

Fig. 10 Antimicrobial activity of *A. mexicana* by well diffusion method **A**: Plate showing zone of inhibition against *E. coli* B: Plate showing zone of inhibition against *Bacillus subtilis* C: Plate showing zone of inhibition against *Staphylococcus aureus* (1) well loaded with gel, incorporated nanoparticles of methanol extra**ct (2)** well loaded with gel, incorporated with nanoparticles of latex **(3)** well loaded with methanol extract **(4)** well loaded with latex **(5)** well loaded with ethyl acetate extract

The increased production of reactive oxygen species and free radicals by silver nanoparticles which in turn causes DNA and cell damage (AshaRani *et al*., 2009). The nanoparticles also interfere in membrane protein synthesis and activate the signaling pathway which reduces the proliferation of cell (Liao *et al*., 2019). The enhanced biological activities of plant-based nanoparticles have also been reported by many authors. There is a tremendous increase in the popularity of using plant-based extracts for synthesis nanoparticles due to advantages like a higher degree of activity, lesser toxicity, greater stability, better yield, less expensive, and many more. The nanoparticles from *A. mexicana* are synthesized which entrap the biologically active ingredients present in the plant. The formulation showed promising antibacterial activity for topical application against both gram positive and negative bacteria. The gel provides a more suitable topical drug delivery approach for the comfort of patient as well as lesser side effects than antibiotics and synthetic drugs which interact with other metabolites of the body. The encapsulated phytoconstituents in the nanoparticles also improves the bioavailability and membrane permeability in the affected area to exert better therapeutic effect. The current formulation will be helpful in facilitating targeted drug delivery, controlled release of drugs, and maximize drug retention time, an innovative approach for topical drug application.

4. CONCLUSION

The targeted drug delivery system, minimum dosage, enhanced efficacy, and minimum side effect became the key criteria for delivering drugs into the body. The nanoparticles from *A. mexicana* are successfully synthesized and incorporated into the gel formulation. The formulated nanoparticulate gel shows a stable and uniform distribution of nanoparticles in the gel. The gel loaded with nanoparticles exhibited good antibacterial efficacy against both gram positive and negative bacteria. Our study presents a novel nanoparticle mediated drug delivery approach, especially for topical drug application. The nanoparticle mediated drug delivery could be used in place of various conventional methods of treatment.

Author contributions. All the authors listed in this article have contributed to the conception, design, analysis and data interpretation.

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Conflict of Interest: The authors declare there is no conflict of interest in any form in this research.

5. REFERENCES

[1]. Abubacker, M. N. and Ramanathan, R. (2012). Antibacterial Activities of Argemone mexicana L. (Papavaraceae) Leaf Extract on Pathogenic Bacterial Strains. Drug Invention Today, 4(7), pp 385-87.

[2]. Alagesaboopathi, C. and Kalaiselvi, N. (2012). Antimicrobial activities of the root, stem and leaf extracts of Argemone mexicana L. International Journal of Biosciences, 2(5), pp 61-68.

[3]. AshaRani, P. V., Grace, L. K. M., Hande, M., P. and Valiyaveettil S. (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. American Chemical Society Nanoscience, 3(2), pp 279-290.

[4]. Bhardwaj, M., Duhan, J. S., and Kumar, A. S. (2012). Antimicrobial potential of Argemone mexicana: An in vitro study. Asian Journal of Microbiology Biotechnology and Environmental Sciences, 14 (2), pp 353-357.

[5]. Brahmachari, G., Roy, R., Mandal, L. C., Ghosh, P. P. and Gorai, D. (2010). A new long-chain secondary alkanediol from the flowers of Argemone mexicana. Journal of Chemical Research, 34(11), pp 656-657.

[6]. Chairuangkitti, P., Lawanprasert, S., Roytrakul, S., Aueviriyavit, S., Phummiratch, D., Kulthong, K. and Maniratanachote, R. (2013). Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways. Toxicology in vitro, 27(1), pp 330-338.

[7]. Chang, Y. C., Hsieh, P. W., Chang, F. R., Wu, R. R., Liaw, C. C., Lee, K. H. and Wu, Y. C. (2003). Two new protopines argemexicaines A and B and the anti-HIV alkaloid 6-acetonyldihydrochelerythrine from formosan Argemone mexicana. Planta medica, 69(02), pp 148-152.

[8]. Chevallier, A. (1996). The encyclopedia of medicinal plants. Dorling Kindersley Publisher, Michigan 1996.

[9]. Dada, A. O., Ojediran, J. O., Dada, F. E., Olalekan, A. P. and Awakan, O. J. (2017). Green synthesis and characterization of silver nanoparticles using Calotropis procera extract. Journal of Applied Chemical Science International, 8(4), pp 137-143.

[10]. Dash, G. K. and Murthy, P. N. (2011). Evaluation of Argemone mexicana Linn. Leaves for wound healing activity. Journal of Natural Product and Plant Resources, 1(1), pp 46-56.

[11]. Swamy, A. V., Gulliaya, S., Thippeswamy, A., Koti, B. C. and Manjula, D. V. (2012). Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. Indian Journal of Pharmacology, 44(1), pp 73.

[12]. Doss, A., Mubaracki, H. M., Vijayasanthi, M. and Venkataswamy, R. (2012). In-vitro antibacterial activity of certain wild medicinal plants against bovine mastitis isolated contagious pathogens. Asian Journal of Pharmaceutical and Clinical Research, 5(2), pp 90-93.

[13]. Fletcher, M. T., Takken, G., Blaney, B. J. and Alberts, V. (1993). Isoquinoline alkaloids and keto-fatty acids of Argemone ochroleuca and A. mexicana (Mexican poppy) seed. I. An assay method and factors affecting their concentration. Australian Journal of Agricultural Research, 44(2), pp 265-275.

[14]. Grzelak, A., Wojewódzka, M., Meczynska-Wielgosz, S., Zuberek, M., Wojciechowska, D. and Kruszewski, M. (2018). Crucial role of chelatable iron in silver nanoparticles induced DNA damage and cytotoxicity. Redox Biology, 15, pp 435-440.

[15]. Haisova, K., and Slavik, J. (1975). On the minor alkaloids from Argemone mexicana L. Collection of Czechoslovak Chemical Communications, 40(5), pp 1576-1578.

[16]. Ibrahim, H. M. (2015). Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. Journal of Radiation Research and Applied Sciences, 8(3), pp 265-275.

[17]. Israilov, I. A. and Yunusov, M. S. (1986). Alkaloids of four species of Argemone. Chemistry of Natural Compounds, 22, pp 189-192.

[18]. Izzo, A. A., Carlo, I., Biscardi, G., Fusco, D., Mascolo, R., Borreli, N., Capasso, F., Fasulo, F. and Autore, M. P. Biological screening of Italian medicinal plants for antibacterial activity. Phytotherapy Research, 9 (4), pp 281-286.

[19]. Jain, R. A., Agarwal, R. C., Dubey, D., Verma, R. and Jain, R. (2012). Evaluation of antibacterial and antioxidant activity of fruits extract of Argemone mexicana Linn. International Journal of Pharmaceutical Innovation, 2, pp 45-51.

[20]. Kaviya, S., Santhanalakshmi, J., Viswanathan, B., Muthumary, J. and Srinivasan, K. (2011). Biosynthesis of silver nanoparticles using Citrus sinensis peel extract and its antibacterial activity. Spectrochimica. Acta, Part A Molecular and Biomolecular Spectroscopy, 79(3), pp 594-8.

[21]. Kumar, P. V., Pammi, S. V. N., Kollu, P., Satyanarayana, K. V. V. and Shameem, U. (2014). Green synthesis and characterization of silver nanoparticles using Boerhaavia diffusa plant extract and their anti-bacterial activity. Industrial Crops and Products, 52, pp 562-566.

[22]. Liao, C., Li, Y. and Tjong, S. C. (2019). Bactericidal and cytotoxic properties of silver nanoparticles. International Journal of Molecular Sciences, 20(2), pp 449.
[23]. Manandhar, N. P. (2002). Plants and people of Nepal. Timber press. USA, Ethnobotany, 2002, pp. 509-511.
[24]. Mashwani, Z. U. R., Khan, T., Khan, M. A. and Nadhman, A. (2015). Synthesis in plants and plant extracts of silver nanoparticles with potent antimicrobial properties: current status and future prospects. Applied Microbiology and Biotechnology, 99 (23), pp 9923-9934.
[25]. Markus, J., Wang, D., Kim, Y. J., Ahn, S., Mathiyalagan, R., Wang, C. and Yang, D. C. (2017). Biosynthesis, characterization, and bioactivities evaluation of silver and gold nanoparticles mediated by the roots of Chinese herbal Angelica pubescens Maxim. Nanoscale Research Letters, 12 (1), pp 46.
[26]. Daryanto, S., Wang, L. and Jacinthe, P. A. (2017). Impacts of no-tillage management on nitrate loss from corn, soybean and wheat cultivation: A meta-analysis. Scientific reports, 7(1), pp 12117.
[27]. Osho, A. and Adetunji, T. (2010). Antimicrobial activity of the essential oil of Argemone mexicana Linn. Journal of Medicinal Plants Research, 4(1), pp 19-22.
[28]. Pandey, A. and Karanwal, V. (2011). A study of extract optimization and effect of metal ions on antibacterial properties of Argemone mexicana. Asian Journal of Plant Science and Research, 1(2), pp 43-48.
[29]. Rahman, M. M., Alam, M. J., Sharmin, S. A., Rahman, M. M., Rahman, A., & Alam, M. F. (2009). In vitro antibacterial activity of Argemone mexicana L (Papaveraceae). CMU J Nat Sci, 8(1), pp 77-84.
[30]. Rosas–Pinon, Y., Mejia, A., Diaz, G., & Ruiz, M. Isabel Aguilar, S. Sanchez–Nieto, and J. Fausto Rivero–Cruz. 2012. Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections. Journal of Ethnopharmacology, 141(3), pp 860-865.
[31]. Rostami-Vartooni, A., Nasrollahzadeh, M. and Alizadeh, M. (2016). Green synthesis of seashell supported silver nanoparticles using Bunium persicum seeds extract: application of the particles for catalytic reduction of organic dyes. Journal of colloid and interface science, 470, pp 268-275.
[32]. Shaukat, S. S., Siddiqui, I. A., Khan, G. H. and Zaki, M. J. (2002). Nematicidal and allelopathic potential of Argemone mexicana, a tropical weed: allelopathic and nematicidal potential of Argemone mexicana. Plant and Soil, 245, pp 239-247.
[33]. Siddiqui, I. A., Shaukat, S. S., Khan, G. H. and Zaki, M. J. (2002). Evaluation of Argemone mexicana for control of root-infecting fungi in tomato. Journal of Phytopathology, 150(6), pp 321-329.
[34]. Singh, S. K., Pandey, V. D., Singh, A. and Singh, C. (2009). Antibacterial activity of seed extracts of Argemone mexicana L. on some pathogenic bacterial strains. African Journal of Biotechnology, 8(24), pp 7077-7081.
[35]. Sintubin, L., De Gusseme, B., Van der Meeren, P., Pycke, B. F., Verstraete, W. and Boon, N. (2011). The antibacterial activity of biogenic silver and its mode of action. Applied microbiology and biotechnology, 91(1), pp 153-162.
[36]. Sukumar, D., Arivudai Nambi, R. and Sulochana, N. (1984). Studies on the leaves of Argemone mexicana. Fitoterapia. 55, pp 352-353.
[37]. Tripathi, P. N., Tripathi, M., Pandey, V. B. and Singh, D. (1999). Alkaloids of Argemone mexicana. Oriental Journal of Chemistry, 15(1), pp 185-186.

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