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Antimicrobial Study of Berberis Aristata Loaded PLGA Nanoparticles

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Abstract—*Bio-medical science is widely used in a variety of fields. It has grown abruptly with the passage of time and with growing technology.This paper presents nanoformulation of berberisaristata (daaruhaldi) nanoparticles by using solvent evaporation method. Methanolic extract of plant was used to nanoformulate the nanoparticles in the range of 35 nm to 100 nm, and its characterization was done using various techniques such as SEM, TEM, XRD and FTIR. PLGA encapsulated nanoparticles shows entrapment efficiency as high as 84.6% and percentage yield comes out to be 54.4. Antimicrobial study were also carried out using various stains of gram positive bacteria (S.aureus and B.pumilus) and gram negative bacteria (E.Coli and Pseudomonas aeruginosa) which shows MIC at 2000ppm for gram positive bacteria (both S.aureus and B.pumilus) and 5000 ppm(E.coli) and 1000 ppm(Pseudomonas aeruginosa) for gram negative bacteria. Other applications such as kinetic study of medicinal nanoparticles, Photochemical activity of given nanoparticles could also be carried out in coming future.*

Keywords: *Nanoparticles, berberisaristata, PLGA, Antimicrobial study.*

Introduction: Berberine is a plant alkaloid in both Ayurvedic and Chinese medicine with a long history of medicinal use. In Hydrastis Canadensis (goldenseal), Coptis chinensis (Coptis or goldenthread), Berberis aquifolium (Oregon grape), Berberis vulgaris (barberry) and Berberis aristata (tree turmeric) it is found. Berberine alkaloid is present in plant stem, roots, rhizomes, and bark. Berberine extracts and decoctions have demonstrated strong antimicrobial activity against a variety of species including bacteria, viruses, fungi, protozoa, helminths, and chlamydia. Berberine is an over-the-counter drug used in China to treat bacterial diarrhea.The hypoglycemic effect of berberine was first recorded in 1988 when berberine was used to diabetic patients to treat diarrhea. In addition, numerous clinical and preclinical trials show Berberine's ameliorative effect on several conditions including metabolic, neurological, and cardiological issues. This analysis offers an overview of the antibacterial activity of berberis aristata loaded nanoparticles. Several literatures have been published by different authors, investigating the phytochemical and medicinal aspects together with conventional applications, but still much literature is not available about the same.Ayurveda health care's primary goal is to restore patients ' physical, psychological, and emotional stability, improving health, preventing disease, and treating any existing disease.There is an unprecedented increase in the number of patients who are seeking medicinal and herbal medicine. Herbal medicines are now in great demand in the developing world for primary health care, not because they are cheap, but also for better cultural acceptance, better functionality of the human body and minimal side effects. Herbal medicine remains the cornerstone of around 75–80% of the world's population, mostly in developing countries with primary healthcare. Nevertheless, for biological activity only 6 percent of the total 250,000-400,000 plant species were studied and approximately 15 percent were examined phytochemically. Studying Berberis aristata's enamorous benefits, it seems necessary to properly nanosynthesize the herbs. So, PLGA encapsulated berberis aristata nanoparticles were synthesized, characterized and further antimicrobiual activity of the synthesized nanoparticles were carried out using various gram positive and gram negative stains of bacteria.

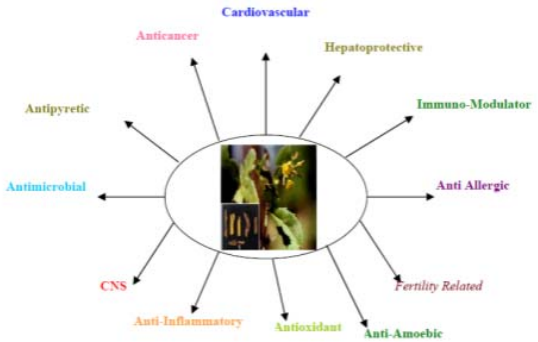

Fig. 1: Pharmacological activity of berberis aristata

MATERIALS AND METHODS

Materials: From the local market, Berberis aristata was purchased. The polymer polymer (D, L-lactide-co-glycolide) (PLGA) was obtained from Sigma-Aldrich with a copolymer ratio of 50:50 (Mw= 24000 to 38000). Surfactants like Pluronic F-68 and Polyvinyl alcohol (PVA)(Mw 30,000-70,000 Da) are procured from Sigma-Aldrich (St. Louis, MO, USA). The analytical grade is considered for the organic reagents and solvents such as acetonitrile, acetone and methanol.

Preparation of Berberis aristata loaded PLGA Nanoparticles by Solvent Evaporation Method:

To achieve a standardized size range, Berberis aristata was powdered and sieved in a blender. This sieved powder has been dissolved in methanol for further extraction. 8 gm of powder has been immersed in 100 ml of methanol for 72 hours. This preparation was then filtered through a filter paper Whatman No.1 to allow the solvent to evaporate from the filtered extract at room temperature. The dried extract thus obtained was stored for further use in bottles and cooled.Nanoparticles have been synthesized using the method of solvent evaporation.Dry extract was 10 mg and PLGA was co-dissolved in 10 ml of organic solvent acetonitrile. The organic step was applied using a 50 watt homogenizer to 25 ml of deionized water containing 0.1% PVA / Pluronic F-68. The solution of nanoparticles was stirred to evaporate acetonitrile for 4 hours. After suspended NP's are centrifuged (REMI, INDIA) for 20 min at 15,000 rpm, washed with deionized water and dried to obtain the dry nanoparticles and processed at 4^0C for further use.

Particle Size and Morphology Characterization: During the study using the Solvent Evaporation Method, PLGA encapsulated Berberis aristata nanoparticles were successfully prepared. The aim is to study the particle size in the aqueous solution where the suspension of nanoparticles was analyzed after removing the organic solvent by continuously using magnetic stirring for at least 4 hours. The results obtained from the formulation F1 SEM and TEM images showed that spherical nanoparticles (prepared by the use of acetonitrile and PluronicF-68 between 60 nm and 100 nm) are shown in fig. (1 and 2)

XRD and FTIR Study: Using X-beam diffractrometer, The XRD patterns of PLGA-loaded nano particles, methanolic extract and PLGA were examined.The estimates were taken at 45 kV voltage and 40 mA anodic current. XRD patterns were acquired at diffraction edges (2) ranging from 0o to 90o at 2o per moment. XRD patterns were performed to determine the nature of the compound formed whether crystalline or amorphous. Furthermore, FTIR experiments were also performed on Berberis aristata charged PLGA nanoparticles to determine whether the polymer interacted with the product during nanoencapsulation.

Determination Of Entrapment Efficiency And Percentage Yeild: The amount of Berberis aristata trapment was determined by the UV analysis (Shimadzu, UV-1800) at 280 nm. UV was monitored at 280 nm wavelength and the corresponding peak absorption was reported after centrifugation and removal of the first pellet. A standard calibration curve was drawn using different concentrations of methanol extract (1-10 mg in 10 ml water) versus maximum supernatant absorption and used to examine the samples in direct determination of encapsulation efficiency and percentage yield, as indicated by UV monitoring.The following equations have been used to calculate the efficiency and percentage yield of encapsulation.

%EE = [(Drug added - Free "unentrapped drug")/Drug added] *100

Percentage Yield= [(Weight of Nanoparticles)/ (Weight of PLGA+ Weight of methanolic Extract)] *100

Antimicrobial activity of Berberis aristata loaded PLGA nanoparticles: When examined against Enterococcus coli, Psedomonas aeruginosa, B.pumilus, and Staphylococcus aureus, the activities and properties of Berberis aristata-loaded nanoparticle. Cup plate method was used to determine the MIC of these test compounds. In the cup plate process, the antimicrobial material diffuses from the cup to some degree through a solidified agar surface in a petri-dish in order to completely inhibit the growth of added microorganism in a circular area or region around the cavity containing the solution of a known quantity of antimicrobial substance. The antimicrobial activity is expressed in millimeters as the zone of inhibition, measured by a zone reader.(The amount of suspended nanoparticles used to study antimicrobial activity). Comparative study was conducted using methanol extract of pure drug along with drug-loaded PLGA nanoparticles. After a 24-hour incubation period at a temperature of 37 C, the plates were then examined to determine the MIC of the whole test compounds as the lowest concentration that could inhibit bacteria's visible growth.

RESULTS AND DISCUSSION

Formulation, Optimization and Characterization Of Nanoparticles

(The volume of nanoparticles suspended for the analysis of antimicrobial activity). Comparative analysis with drug-loaded PLGA nanoparticles was performed using methanol extract of pure material. The plates were then analyzed after a 24-hour incubation period at a temperature of 37 C to assess the MIC of the entire test compounds as the lowest concentration that could prevent the visible growth of the bacteria.

Table 1: Characteristics of drug loaded PLGA nanoparticles:

Formulation	Stabilizer	Solvent	Drug-Polymer ratio	Process Yield (%)	Encapsulation Efficiency (%)
F-1	Pluronic F-68	Acetonitrile	1:5	16.66	92.64
F-2	PVA	Acetonitrile	1:5	37.28	85.73
F-3	Pluronic F68	Acetone	1:5	66.13	95.8
F-4	PVA	Acetone	1:5	46.5	58.7

It was inferred from Table 1 that the percentage yield of formulations F-1 and F-3 was moderate, but the efficiency of drug encapsulation was high in formulation F-1. Therefore, using F-1, various parameters such as morphological analysis, length, and in vitro release of drugs and antimicrobial test were performed.

The Particle size and Morphological analysis: During the study using the Solvent Evaporation Method, PLGA encapsulated Berberis aristata nanoparticles were successfully prepared. The aim is to study the particle size in the aqueous solution where the suspension of nanoparticles was analyzed after removing the organic solvent by continuously using magnetic stirring for at least 4 hours. The results obtained through the formulation F1, SEM and TEM images showed that the spherical nanoparticles (prepared by the use of acetonitrile and PluronicF-68 ranging from 60 nm to 100 nm) are shown in Fig.(1 and 2).

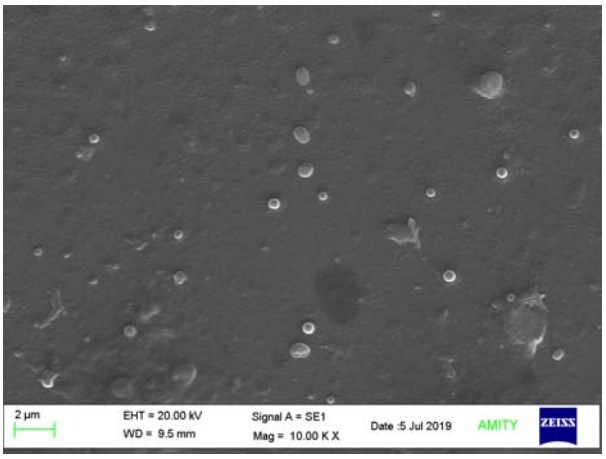

Figure 1 - SEM images of Arjunaterminalia-loaded spherically shaped PLGA nanoparticles

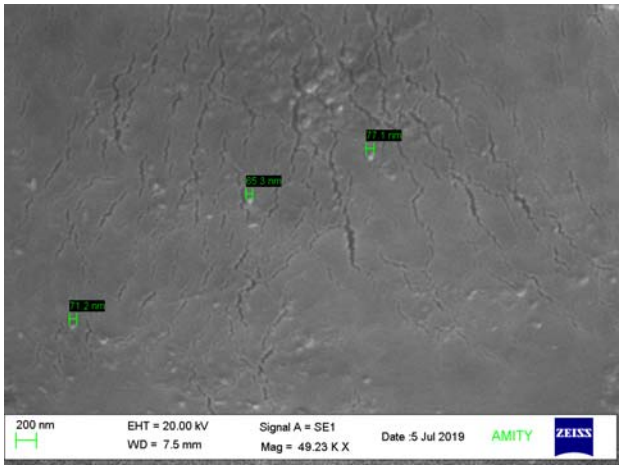

Figure 2 - TEM images of Arjunaterminalia-loaded spherically shaped PLGA nanoparticles showing particle size in between 60 nm to 100nm

XRD And FTIR Studies: A strong band at 3227 cm-1 is reported due to the stretching mode of N-H (Aromatic Amine vibration). In the creation and composition of biological systems, amine bonds play a major role, representing, for example, the major chemical bonds that connect amino acid building blocks together to provide proteins. A strong band at 1688 cm-1 is recorded due to Stretching mode C = C (alpha-beta-unsaturated ketone)A Low peak at 1443 cm-1 is due to stretching mode C-N (Sulfate / Primary Amine), 1278 cm-1 due to stretching mode C-N Aromatic Amine, 1043 cm-1 due to stretching C = O and a very low peak at 2936 cm-1 due to stretching mode C-H (Alkene)

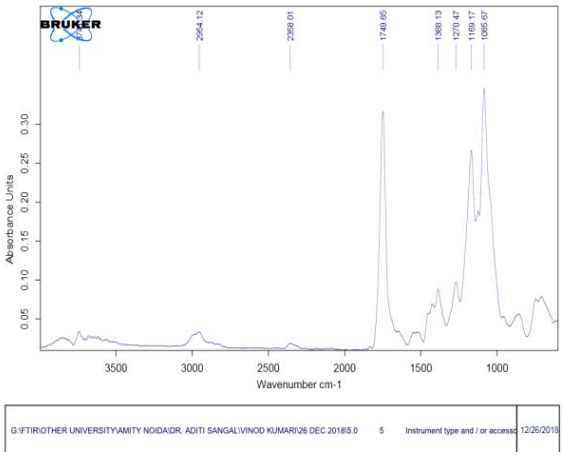

Fig. 3(a) FTIR PLGA

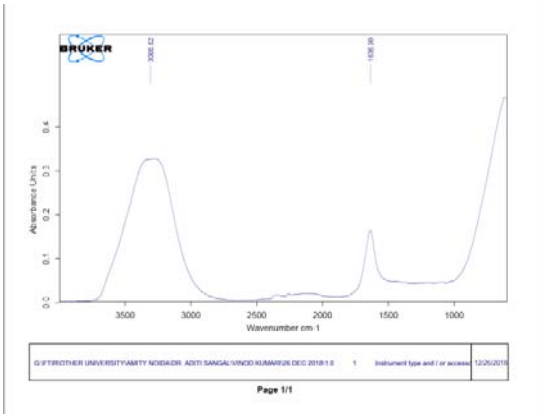

Fig. 3(b) FTIR PLGA encapsulated berberis aristata nanoparticles

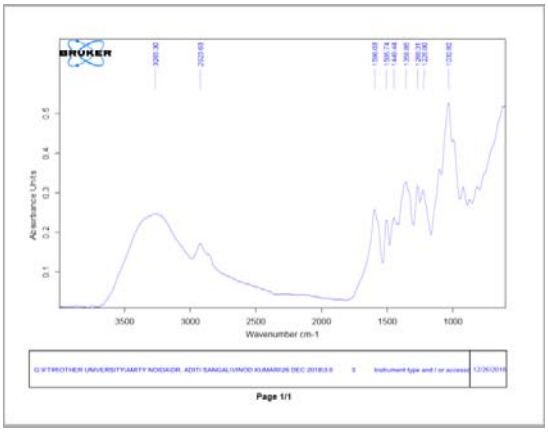

Fig. 3(c) FTIR methanolic extract of berberis aristata nanoparticles

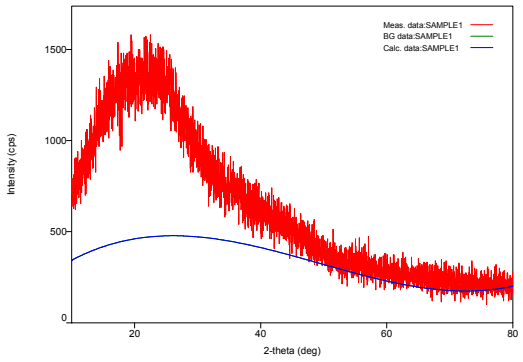

Fig. 3(d) The XRD spectra for the drug loaded polymeric nanoparticles

The XRD spectra for the drug loaded polymeric nanoparticles were obtained Fig. 3(d) and indicated that there is no characteristic peaks were observed, possibly due to the amorphous nature of the drug**.**

Entrapment Efficiency: The entrapment efficiency of Berberis aristata loaded PLGA nanoparticles for Berberis aristata active compound was found to be 82.34%. Strongly depends on the drugs that were about to be encapsulated to select the method used in the production of nanoparticles.

Antimicrobial activities study: The MIC of Berberis aristata-loaded nanoparticles against B.pumilus and Staphylococcus aureus shows MIC in the range of 2000ppm whereas it shows MIC in the range of 1000ppm for gram negative bacteria such as E.coli and Pseudomonas aeruginosa. The efficacy of nanoparticles during the inhibition of bacterial and fungal growth was attributable to the penetration of nanoparticles in bacterial and fungal cells and the comparatively improved distribution of the Berberis aristata at the site. The use of nanoparticles to trap antimicrobial hydrophobic compounds could somehow boost the activity due to 3 factors i.e. sustained release, improved hydrophilicity and better penetration due to small scale. Table 2 lists the MIC with various gram-positive and gram negative bacteria and the inhibition zone obtained, and the zones obtained are shown in Figure 5 (a, b, c.d)

Table 2: Various zone of inhibition obtained against different concentration for different bacterial stains.

S. No	Bacteria	Mic(In Ppm)	Zone Of Inhibition (Np)	Bacteria Stain
1	Staphylococcus Aureus	10000	18.54	Gram Positive Bacteria
2		8000	16.11	Gram Positive Bacteria
3		4000	13.09	Gram Positive Bacteria
4		2000	6.47	Gram Positive Bacteria
5		1000	No Zone Of Inhibition	Gram Positive Bacteria
6	B.Pumilus	10000	19.47	Gram Positive Bacteria
7		8000	17.25	Gram Positive Bacteria
8		4000	14.30	Gram Positive Bacteria
9		2000	7.45	Gram Positive Bacteria

10		1000	No Zone Of Inhibition	Gram Positive Bacteria
1	E.Coli	10000	21.47	Gram Negative Bacteria
2		8000	18.13	Gram Negative Bacteria
3		6000	14.10	Gram Negative Bacteria
4		5000	12.51	Gram Negative Bacteria
5		2000	No Zone Of Inhibition	Gram Negative Bacteria
6	Pseudomonas Aeruginosa	10000	21.05	Gram Negative Bacteria
7		80000	18.07	Gram Negative Bacteria
8		4000	14.25	Gram Negative Bacteria
9		1000	11.10	Gram Negative Bacteria
10		800	No Zone Of Inhibition	Gram Negative Bacteria

CONCLUSION

Solvent evaporation method shows less extensive, easier, less energy consumption and is a commonly used method for the production of spherical nanoparticles, especially without additives. Using solvent evaporation process, a number of formulations with different combinations of surfactants, medications, polymer and volumes are prepared. Our result shows that using solvent evaporation will substantially improve the efficiency of encapsulation i.e.82.3 percent, particle size less than 100 nm.. Nanoparticles ' antimicrobial activitiesare tested against gram positive and gram negative bacterias along with MICs ranging from 1000 ppm to 15,000 ppm. Antimicrobial results show that nanoparticles of this type are certainly more effective in the growth of bacterial inhibition.

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REFERENCES

[1] Ahmeda T, Gilanib AH, Abdollahid M, Dagliae M, Nabavif SF, Nabavif SM. Berberine and neurodegeneration: A review of literature. Pharmacol Repo 2015;67:970–979.

[2] Watt G. A dictionary of the economic products of India. Published under the authority of His Majesty's Secretary of State for India in Council, Kolkatta. London: Yohn Murry 1889:652.

[3] Kirtikar KR, Basu BD. Indian medicinal plants. Allahabad: LM Basu Publica 1933:2422.

[4] Anonymous. The wealth of India. Publications and Information Directorate CSIR, New Delhi 1985;1:176–179.

[5] Rajasekaran A, Kumar N. Rasont—a traditional crude drug prepared from Berberis sp. and its uses. Indian J Tradit Know 2009;8:562–563.

[6] Ray R, Gururaja KV, Ramchandra TV. Predictive distribution modeling for rare Himalayan medicinal plant Berberis aristata DC. J Environ Biol 2011;32:725-730 .

[7] Shenoy PKR, Yoganarasimhan SN. Evaluation of antibacterial activity of Elanir kujambu—an Ayurvedic eye formulation. Indian J Tradit Know 2009;8:272–274.

[8] Srivastava SK, Khatoon S, Rawat AKS, Mehrotra S, Pushpangadan P. Pharmacognostic Evaluation of the Root of Berberis aristata DC. Nat Prod Sci 2001;7:102–106.

[9] Parmar C, Kaushal MK. Berberis aristata. In: Wild Fruits. Kalyani Publishers, New Delhi, India 1982:10–14 (http://www.hort.purdue.edu/newcrop/parmar/03.html)

[10] Ambastha SP. The Wealth of India. Publication and Information Directorate, New Delhi, CSIR 1988;2:118.

[11] Chander, V., Aswal, J. S., Dobhal, R., & Uniyal, D. P. (2017). A review on Pharmacological potential of Berberine; an active component of Himalayan Berberis aristata. *J Phytopharmacol*, *6*(1), 53-58.

[12] Kumari, V., & Sangal, A. (2019). Study of Antimicrobial activity of Berberis aristata loaded poly (DL-lactide-co-glycolide) nanoparticles. *Research Journal of Pharmacy and Technology*, *12*(2), 499-507.

[13] Kumari, V., & Sangal, A. (2018). Preparation, Characterization and Optimization of Cinnamon-Loaded PLGA Nanoparticles. In *Green Chemistry in Environmental Sustainability and Chemical Education* (pp. 219-224). Springer, Singapore.

[14] Kumari, V., & Sangal, A. (2019). Synthesis, Characterization, Antimicrobial activity and Release Study of Cinnamon loaded poly (DL-lactide-co-glycolide) Nanoparticles. *Research Journal of Pharmacy and Technology*, *12*(4), 1529-1535.