Anti-Inflammatory Activity Of Embelia Ribes Derived Herbal Nanoformulations And Its Mediated Silver Nanoparticles: An Invitro Study

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ABSTRACT

Background : The aim of this study is to determine the anti-inflammatory activity of Embelia ribes mediated silver nanoparticles in an in-vitro model in different concentrations.

Materials and Methods

To prepare the solution, 1 mL of Embelia ribes extract was combined with 99 mL of distilled water and allowed to mix for 30 minutes. Subsequently, silver nitrate was introduced into the mixture, and it was placed in an orbital shaker equipped with a magnetic stirrer for a duration of 2 hours. The solution's color change was monitored over the course of 3 days. Following this, centrifugation was employed to isolate the silver nanoparticles. The evaluation of anti-inflammatory activity was conducted through the Egg Albumin Assay and Albumin Denaturation using Bovine serum.

Results

The presence of a peak at 650 nm in the UV-visible spectroscopy results signifies the successful formation of silver nanoparticles. The investigation has substantiated that an escalation in the concentration of silver nanoparticles synthesized through Embelia ribes leads to a dose-dependent anti-inflammatory response. Notably, the Embelia ribes-mediated silver nanoparticles demonstrated their highest level of anti-inflammatory activity, achieving a remarkable 78% inhibition rate when administered at a dosage of 50 µL.

Conclusion

Silver nanoparticles synthesized through Embelia ribes exhibit noteworthy anti-inflammatory properties. Nanoparticles derived from herbal sources additionally present reduced toxicity .

Keywords: Anti-inflammatory,Embelia ribes, Egg Denaturation Assay, Bovine Denaturation assay, Silver nanoparticles.

Introduction

The increasing awareness of the potential side effects associated with conventional medicaments has led to a growing interest in traditional herbal medicines. (Althumairy, Teixeira and Diogenes, 2014).Herbal remedies have been used for ages in various traditional medical systems, including Ayurvedic, Chinese, Vietnamese, and Tibetan practices. (Wangchuk, 2018)They have been employed to treat a range of conditions, such as diabetes, inflammation, intestinal worms, and dental and skin diseases mainly because of their least side effects.(Dutta, Goswami and Mitra, 2021)

Embelia ribes Burm F, a medicinal woody climber belonging to the Myrsinaceae family, stands out as a pivotal herb. (Hossan *et al.*, 2018) It boasts a storied history of use dating back to ancient times, where it has been employed to address a wide array of ailments(Radhakrishnan, Gnanamani and Mandal, 2011; Alsamhary, 2020). In the realm of Indian Ayurvedic Practice, it is affectionately known as "false black pepper" or "Viidanga." Acknowledging its paramount importance, the Medicinal Board of the Government of India, based in New Delhi, has designated E. ribes as one of the 32 medicinal plant species crucial for large-scale cultivation, owing to its multifaceted medicinal applications as outlined in "Agro-techniques of Selected Medicinal Plants." This herb finds extensive application in 75 different Ayurvedic preparations, with its fruits, seeds, leaves, and roots harnessed to treat diverse diseases, primarily due to the presence of phenolic compounds such as Embelin.

Embelin, a quinone extracted in an aqueous or ethanolic form from the fruit, seeds, and stem of E. ribes, exhibits various beneficial properties as quinones are known for its biological and color stability. I(Jagtap *et al.*, 2022)t is said to have antibacterial,antioxidant (Radhakrishnan, Gnanamani and Mandal,

2011) antifertility, antiprotozoal, anti-inflammatory, and antioxidant effects.(Lal and Mishra, 2013)(Lal and Mishra, 2013; Jagtap *et al.*, 2023)

Nanoparticles play a pivotal role in modern science and offer a multitude of advantages. Nanoparticles, characterized as clusters of atoms ranging in size from 1 to 100 nm, often exhibit enhanced physical, chemical, and biological properties when compared to their macro-scale counterparts. (Oberdörster, Stone and Donaldson, 2007) Notably, silver bio-nanoparticles have been found to possess inhibitory and bactericidal effects making them of significant commercial and scientific interest due to their unique catalytic, optical, and disinfectant properties. (Alsamhary, 2020) Due to their smaller size, nanoparticles offer a substantially larger surface area that comes into contact with bacterial walls, leading to membrane disruption and DNA damage. (Rosli, Teow and Mahmoudi, 2021) Nanoparticles can be obtained in different shapes depending upon their area of application. However, conventional methods of synthesizing silver nanoparticles, such as physical or chemical approaches including sol-gel processes, micelle formation, chemical precipitation, hydrothermal methods, pyrolysis, and chemical vapor deposition, have raised concerns regarding toxicity and environmental hazards. (Hachem *et al.*, 2022)

To overcome these issues, green synthesis of silver nanoparticles has emerged as a new area of research and medicine (Cao, 2017)This method offers potential benefits to human health with minimal or no side effects.(Abd-Elsalam, 2021) The current research is focused on exploring the anti-inflammatory properties of silver nanoparticles synthesized using Embelia ribes seeds as a green and natural source. (Das *et al.*, 2019)The objective is to develop these nanoparticles as a valuable addition to the field of medicine, potentially providing safe and effective anti-inflammatory effects.(Manikandan *et al.*, 2015)

Therefore this study is conducted to evaluate the anti inflammatory potential of Silver nanoparticles mediated Embelia ribes and doo further investigation for its therapeutic use in the field of dentistry .(Shanmugam *et al.*, 2021)

Materials And Methods

The in vitro studies were conducted in the nanobiotechnology lab of Saveetha Dental College and Hospitals ,Chennai.

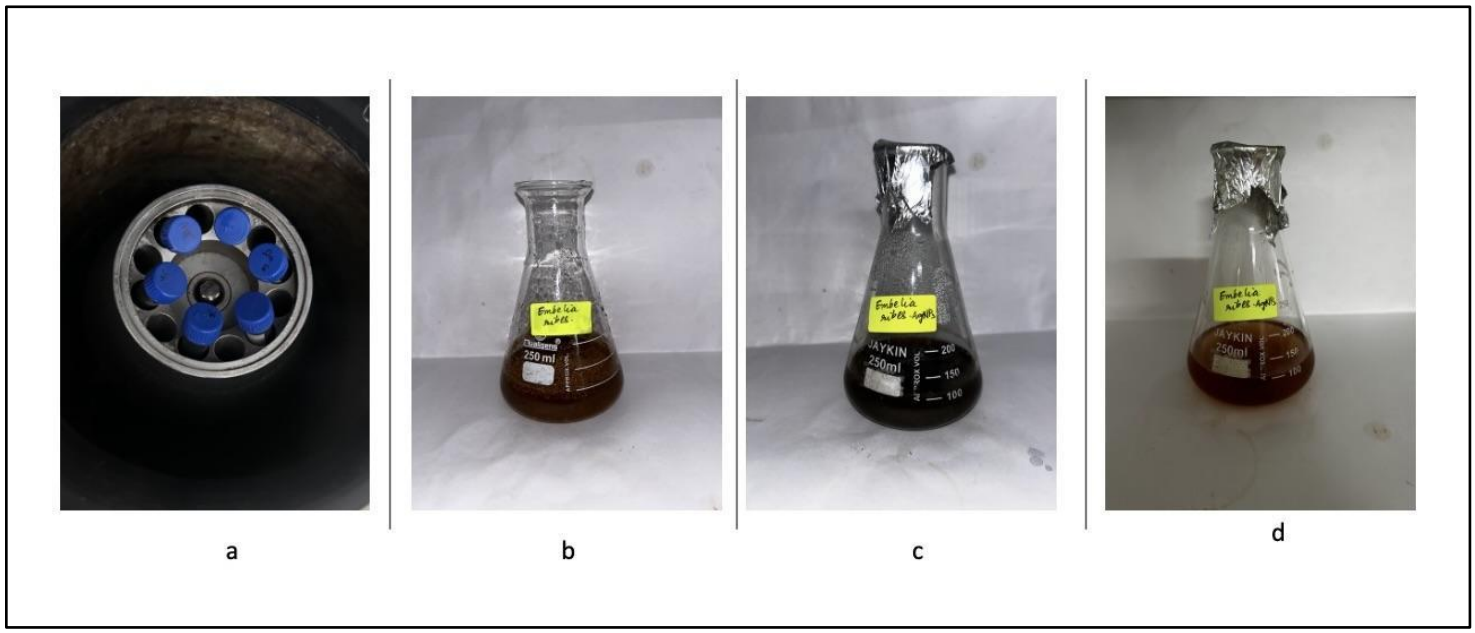
Preparation of Plant Extract

Seeds of Embelia ribes were acquired from an online herbal company. The chemical DPPH was obtained from Sigma Aldrich, India. Hen's eggs were used for the albumin denaturation assay. Silver nitrate solution was employed to synthesize silver nanoparticles (Zhang *et al.*, 2019; Shanmugam *et al.*, 2021). The obtained seeds were transformed into a powdered state. Subsequently, one gram of E. ribes powder was blended with 100 ml of distilled water within a beaker and subjected to boiling for a duration of 10-20 minutes using a heating mantle.(Narendra *et al.*, 2020) Following the boiling process, the resulting extract was filtered through Whatman filter paper (Figure 2).

Synthesis of Silver Nanoparticles

To synthesize nanoparticles, a solution containing 1 mMol of silver nitrate in 99 ml was prepared. (Abd-Elsalam, 2021) Subsequently, 10 ml of the filtered E. ribes extract was introduced into this

solution (Figure3) and the mixture was positioned on an orbital shaker equipped with a magnetic stirrer for a duration of 2 hours. (Nagarajan *et al.*, 2019)The color change was monitored at 24-hour intervals over a period of 3 days.(figure 4) Following this observation period, the extract underwent centrifugation at a speed of 8000 rpm for 10 minutes, resulting in the formation of silver nanoparticles.(Figure 1)(Alsamhary, 2020)(Kandhan *et al.*, 2019)



[Figure 1a: Centrifugation for Nano Particle formation and Crude Extract of Embelia Ribes b: Seed extract of Embelia Ribes c: Embelia ribes mediated silver nanoparticles d: Embelia Ribes mediated Silver Nanoparticles and Change in Color after 3 days,confirming formation of SNPs using UV-Visible spectroscopy]

Anti-Inflammatory Activity

The anti-inflammatory activity was assessed using two assays-

1.Albumin Denaturation Assay using bovine serum albumin in a 1% aqueous solution.

2.Egg Albumin Denaturation using hen's egg.

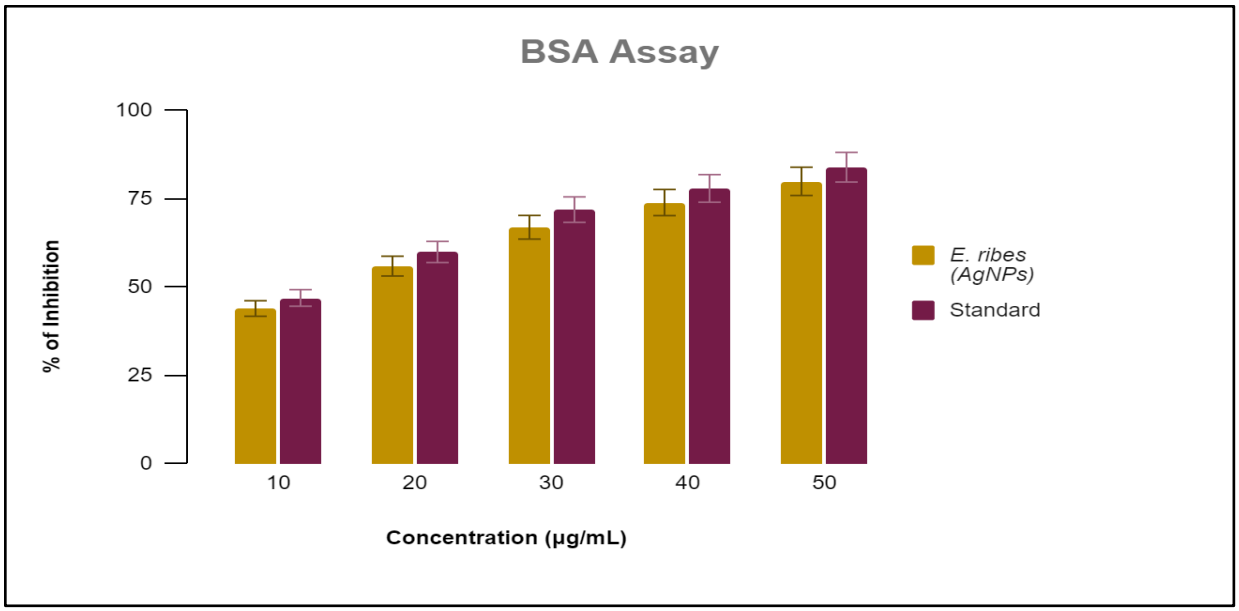
Diclofenac sodium was used as the standard comparison group. Both the plant extract and silver-mediated extracts were added at different concentrations to five different test tubes. Two sets of test tubes were labeled from 10 µL, 20µL , 30µL,40µL and 50 µL. Bovine and egg serum were added to two test tubes along with distilled water as control.

Based on the marked measurements, the prepared silver nanoparticles were added to the five test tubes and left for 10 minutes. Afterward, they were transferred to a hot water bath for an additional 10 minutes. The absorbance of the end product was then measured using a spectrophotometer.

Results

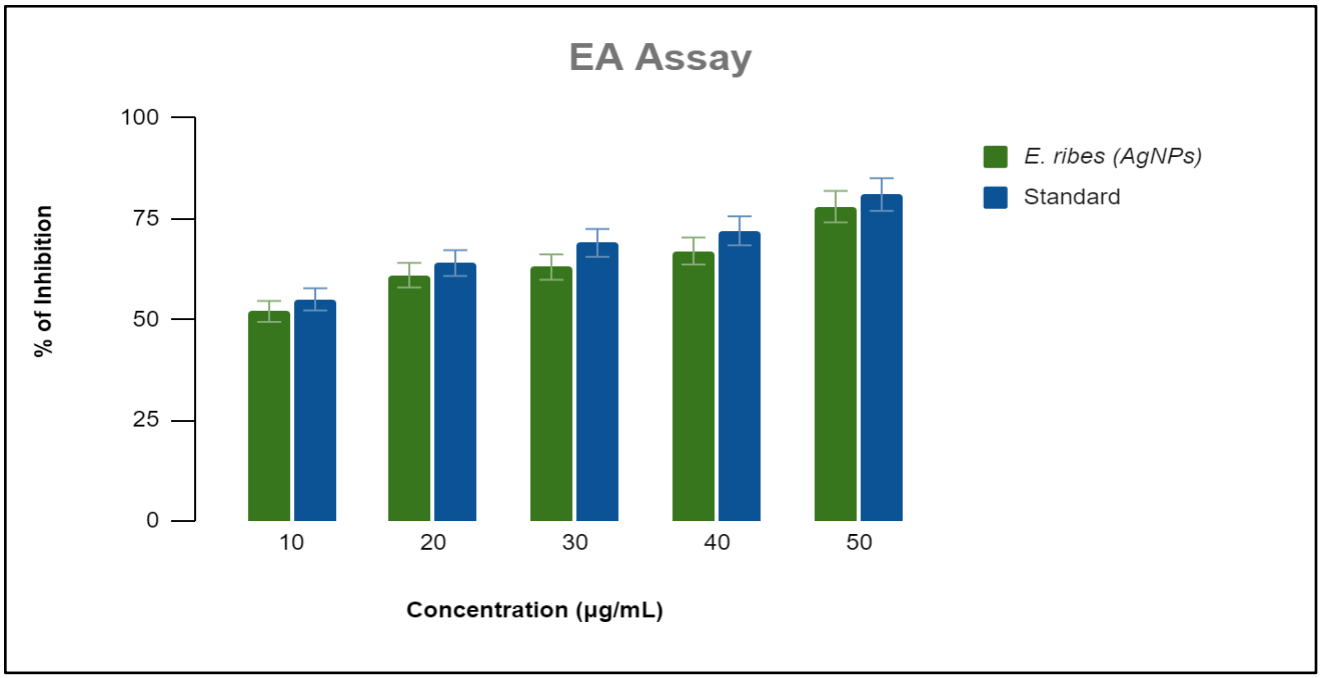
The following graph shows the percentage of absorbance obtained from spectrophotometer-

Graph 1: Antiinflammatory activity of Embelia ribes mediated silver nanoparticles using Bovine Serum



Inference : X-axis shows the concentration used and Y- axis shows the percentage of inhibition in µL. With increase in the concentration of the Embelia ribes mediated silver nanoparticle, increased anti inflammatory activity was observed.Maximum effect was noted at 78% at 50**µ**l concentration for BSA Assay

Graph 2 : Antiinflammatory activity of Embelia ribes mediated silver nanoparticles using Egg Albumin Assay



Inference : X-axis shows the concentration used and Y -axis shows the percentage of inhibition in µL. With increase in the concentration of the Embelia ribes mediated silver nanoparticle, increased anti inflammatory activity was observed.Maximum effect was noted at 77% at 50**µ**l concentration for EA Assay

The Bar Graph (Graph 1) was plotted against different concentrations (10**µ**l, 20**µ**l, 30**µ**l, 40**µ**l, 50**µ**l), % of inhibition .For Bovine Serum Assay the different concentration and antiinflammatory noted are given in table 1.

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Concentration	**Anti-inflammatory Activity (%)**
10 **µ**l	44
20 **µ**l	53
30 **µ**l	61
40 **µ**l	66
50 **µ**l	78
Control	82

Table 1 .Anti Inflammatory effect evaluation of E.ribes mediated AgNps using Bovine Serum Assay.

Similar results were noted with Egg Albumin Assay.The maximum Inhibitory effect was seen 77% at concentration of 50**µ**l,(Rajeshkumar and Malarkodi, 2014)

Discussion

With the increasing side effects of the contemporary medication,herbal medications have now become more active and searched, as they have minimal to no side effects.(Srivastava, 2016)Gardea -Torresdey et al first demonstrated the synthesis of plant based metal nanoparticles using *Alfalfa roots* .Surface plasmon Resonant attribute of the silver nanoparticles have demonstrated agglomeration and interaction at the molecular level with the live cell and its interaction at the molecular level makes it an effective mediator . (Okafor *et al.*, 2013)Silver nanoparticles are known for their unique optoelectronic

properties which enables and facilitates its different nanosize variations in shapes tuned to illumination.(Rafique *et al.*, 2017)

The presence of active metabolites in the seeds of Embelia ribes make it an interest of medicinal importance. Embelin (IUPAC name 2,5 dihydroxy-3-undecyl-1,4 benzoquinone) is an essential metabolite which has anti-inflammatory, analgesic,anticancer ,contraceptive ,anti-infective,antihyperglycemic and wound healing property.(Radhakrishnan, Gnanamani and Mandal, 2011)The other important chemical compounds isolated from the seeds are embeliol, embelinol and embeliaribyl ester .Other chemical components such as essential oils ,alkaloids,phenols and flavionoids boost the medicinal properties of E.ribes.(Jagtap *et al.*, 2023)

In the above study Green synthesis of Silver nanoparticles was prepared using Embelia ribes seeds and the color change was noted for three days and confirmed using UV-vis spectrophotometer analysis.After the effective color change centrifugation (figure 1) was done to obtain the end nanoparticles.Ultraviolet-to-visible (UV-Vis) Spectrophotometer results give final confirmation of the formation of silver nanoparticles showing peak between 400-440nm. The seed extract was used as a capping and a reducing agent and was a cost-effective, simple and eco friendly technique to extract the same.(Souravi, Division of Plant Genetic Resources, Indian Institute of Horticultural Research, Bangalore and Rajasekharan, 2014; Dhayalan *et al.*, 2017)] Green synthesis of the silver nanoparticles also confers stability and better efficacy , and eliminates precipitation of heavy metals.(Rafique *et al.*, 2017) Therefore better therapeutic results are noted with reduced cytotoxicity. The BSA assay and EA assay was used to determine the anti inflammatory activity of E.ribes mediated SNPs when added in

varying concentrations. The control used was sodium Diclofenac which is the gold standard for measuring anti inflammatory activity.(Graph 1 and 2)(*Website*, no date)

The E.ribes mediated silver nanoparticles showed a dose dependent anti-inflammatory effect. At the highest concentration, 50 **µ**l showed 78% of inhibition for Bovine Serum Assay and 77% at 50**µ**l concentration for Egg Albumin Assay.Anti Inflammatory activity of Embelia ribes mediated silver nanoparticles almost has equal effect to the standard drug used which is diclofenac.These nanoparticles displayed strong anti-inflammatory effects at lower concentrations, while their antioxidant activity was more prominent at higher concentrations. This may be attributed to the dose-dependent nature of cellular responses, where different doses elicit varying biological effects.

The present study demonstrated a very simple, faster ,feasible , and eco-friendly synthesis of AgNPs. Silver nanoparticles offer an unwavering advantage in different fields and have proven to be effective in bio-nano-medication. (Aafreen *et al.*, 2019)Studies have shown that AgNPs suppress inflammatory events in the early phase of inflammation. (Zhang *et al.*, 2014).Velmurgan et al illustrated synthesis of silver nanoparticles using peanut shell which demonstrated to have anti fungal and antimicrobial activity.

Herbal Medicine has been used since time memorial in Indian and other ayurvedic practices to cure various diseases.(Zeeshan, Barkat and Mahmood, 2018) Unlike allopathy which in most instances only curbs rather than curing the disease, herbal medicines have shown to eradicate the disease from the root cause with bare minimum side effects. Embelia ribes have shown to have analgesic, antihelminthic, anticancer, anti inflammatory,wound healing and anticancer activity. (Dhayalan *et al.*, 2017; Zeeshan,

Barkat and Mahmood, 2018)

It is essential to consider the limitations of this study, particularly its in-vitro approach. Therefore, caution should be exercised when applying these findings on anti-inflammatory activities to potential clinical outcomes. To gain a more thorough understanding of their therapeutic potential, further research, including clinical trials, is necessary.

Conclusion

The study shows that silver nanoparticles (AgNPs) combined with *Embelia ribes* extract have notable anti-inflammatory effects, indicating their potential as an alternative herbal medication to current therapeutic choices . Their natural origin and effectiveness open up possibilities for further research into their use in healthcare, potentially leading to the development of new therapeutic options.Additionally, these findings underscore the importance of exploring plant-based nanomaterials in modern medicine. With further research, these nanoparticles could contribute to safer and more sustainable therapeutic solutions.

Conflict of Interest

The author declares no conflict of interest

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nil

References

Aafreen, M.M. *et al.* (2019) 'Anti-inflammatory activity of silver nanoparticles prepared from ginger oil—an invitro approach', *Indian journal of public health research and development*, 10(7), p. 145.

Abd-Elsalam, K.A. (2021) *Green Synthesis of Silver Nanomaterials*. Elsevier.

Alsamhary, K.I. (2020) 'Eco-friendly synthesis of silver nanoparticles by and their antibacterial activity', *Saudi journal of biological sciences*, 27(8), pp. 2185–2191.

Althumairy, R.I., Teixeira, F.B. and Diogenes, A. (2014) 'Effect of Dentin Conditioning with Intracanal Medicaments on Survival of Stem Cells of Apical Papilla', *Journal of endodontics*, 40(4), pp. 521–525.

Cao, H. (2017) *Silver Nanoparticles for Antibacterial Devices: Biocompatibility and Toxicity*. CRC Press.

Das, A. *et al.* (2019) 'Anti-inflammatory activity of turmeric oil mediated silver nanoparticles', *Journal of advanced pharmaceutical technology & research*, 12(7), p. 3507.

Dhayalan, M. *et al.* (2017) 'In vitro antioxidant, antimicrobial, cytotoxic potential of gold and silver nanoparticles prepared using Embelia ribes', *Natural product research*, 31(4), pp. 465–468.

Dutta, J., Goswami, S. and Mitra, A. (2021) *Multidimensional Approaches to Impacts of Changing Environment on Human Health*. CRC Press.

Hachem, K. *et al.* (2022) 'Methods of chemical synthesis in the synthesis of nanomaterial and nanoparticles by the chemical deposition method: A review', *BioNanoScience*, 12(3), pp. 1032–1057.

Hossan, M.S. *et al.* (2018) 'Antiviral activity of Embelia ribes Burm. f. against influenza virus in vitro', *Archives of virology*, 163(8), pp. 2121–2131.

Jagtap, R.R. *et al.* (2022) 'Biofabrication of Silver Nanoparticles (AgNPs) Using Embelin for Effective Therapeutic Management of Lung Cancer', *Frontiers in nutrition*, 9, p. 960674.

Jagtap, R.R. *et al.* (2023) 'Embelin isolated from Embelia ribes derived silver nanoparticles and its application in breast cancer nanomedicine', *Materials Today*, 73, pp. 403–411.

Kandhan, T.S. *et al.* (2019) 'Green synthesis of Rosemary oleoresin mediated silver nanoparticles and its effect on Oral pathogens', *Journal of advanced pharmaceutical technology & research*, 12(11), p. 5379.

Lal, B. and Mishra, N. (2013) 'Importance of Embelia ribes: An update', *International journal of pharmaceutical sciences and research*, 4(10), p. 3823.

Manikandan, R. *et al.* (2015) 'Biosynthesis of silver nanoparticles using ethanolic petals extract of Rosa indica and characterization of its antibacterial, anticancer and anti-inflammatory activities', *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*, 138, pp. 120–129.

Nagarajan, K. *et al.* (2019) 'Antioxidant activity of cumin oil mediated silver nanoparticles', *Pharmacognosy journal*, 11(4), pp. 787–789.

Narendra, S. *et al.* (2020) 'Anti-inflammatory activity of nutmeg oleoresin mediated silver nanoparticles- An In- vitro study', *Biomedicine (Trivandrum)*, 39(2), pp. 234–238.

Oberdörster, G., Stone, V. and Donaldson, K. (2007) 'Toxicology of nanoparticles: A historical perspective', *Nanotoxicology*, 1(1), pp. 2–25.

Okafor, F. *et al.* (2013) 'Green synthesis of silver nanoparticles, their characterization, application and antibacterial activity', *International journal of environmental research and public health*, 10(10), pp. 5221–5238.

Radhakrishnan, N., Gnanamani, A. and Mandal, A.B. (2011) 'A potential antibacterial agent Embelin, a natural benzoquinone extracted from Embelia ribes', *Early pregnancy: biology and medicine: the official journal of the Society for the Investigation of Early Pregnancy*, 3(2), pp. 1–7.

Rafique, M. *et al.* (2017) 'A review on green synthesis of silver nanoparticles and their applications', *Artificial cells, nanomedicine, and biotechnology* , 45(7), pp. 1272–1291.

Rajeshkumar, S. and Malarkodi, C. (2014) 'In Vitro Antibacterial Activity and Mechanism of Silver Nanoparticles against Foodborne Pathogens', *Bioinorganic chemistry and applications*, 2014, p. 581890.

Rosli, N.A., Teow, Y.H. and Mahmoudi, E. (2021) 'Current approaches for the exploration of antimicrobial activities of nanoparticles', *Science and Technology of Advanced Materials*, 22(1), pp. 885–907.

Shanmugam, R. *et al.* (2021) 'Curcumin-Chitosan Nanocomposite Formulation Containing -Mediated Silver Nanoparticles, Wound Pathogen Control, and Anti-Inflammatory Potential', *BioMed research international*, 2021, p. 3091587.

Souravi, K., Division of Plant Genetic Resources, Indian Institute of Horticultural Research, Bangalore and Rajasekharan, P.E. (2014) 'Ethnopharmacological Uses of Embelia ribes Burm. F. - A Review', *IOSR journal of pharmacy and biological sciences*, 9(3), pp. 23–30.

Srivastava, S.K. (2016) *Commercial Use of Biodiversity: Resolving the Access and Benefit Sharing Issues*. SAGE Publications India.

Wangchuk, P. (2018) 'Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine', *Journal of biologically active products from nature*, 8(1), pp. 1–20.

Website (no date). Available at: MDPI and ACS Style Salve, P.; Vinchurkar, A.; Raut, R.; Chondekar, R.; Lakkakula, J.; Roy, A.; Hossain, M.J.; Alghamdi, S.; Almehmadi, M.; Abdulaziz, O.; et al. An Evaluation of Antimicrobial, Anticancer, Anti-Inflammatory and Antioxidant Activities of Silver Nanoparticles Synthesized from Leaf Extract of Madhuca longifolia Utilizing Quantitative and Qualitative Methods. Molecules 2022, 27, 6404. https://doi.org/10.3390/molecules27196404.

Zeeshan, U., Barkat, M.Q. and Mahmood, H.K. (2018) 'Phytochemical and antioxidant screening of Cassia angustifolia, Curcuma zedoaria, Embelia ribes, Piper nigrum, Rosa damascena, Terminalia belerica …', *Matrix Sci Pharma* [Preprint]. Available at:

https://matrixscpharma.com/archives/2msp2018/2msp2018-15-20.pdf.

Zhang, S. *et al.* (2014) 'Silver nanoparticle-coated suture effectively reduces inflammation and improves mechanical strength at intestinal anastomosis in mice', *Journal of pediatric surgery*, 49(4), pp. 606–613.

Zhang, Z. *et al.* (2019) 'Green synthesis of silver nanoparticles from mitigates cisplatin-induced nephrotoxicity via down-regulating apoptotic pathway in rats', *Artificial cells, nanomedicine, and biotechnology* , 47(1), pp. 3212–3221.