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A in Vitro Study on Picrorhiza Kurroa Root Mediated Synthesis of Silver Nanoparticles and its Anti Inflammatory Activity

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Abstract

Introduction: *Picrorhiza kurroa is an endangered medicinal herb found in the Himalayas between 3000 and 5000 meters above mean sea level.Due to their multi-target strategy, silver nanoparticles (AgNPs), which may be produced utilizing physical, chemical, or biological (green synthesis) techniques, have displaced all other metal nanoparticles.*

Materials And Methods: *Silver nanoparticles picrorhiza sample was prepared (picrorhiza + AgNP) sample is prepared and kept in the shaker, Egg albumin and bovine serum albumin assays were done and the anti inflammatory was analyzed.*

Results: *The sample showed good anti-inflammatory activity when compared to the standard (diclofenac sodium) and this study provides a less toxic, eco-friendly cost effective way of synthesizing and utilization of silver nanoparticles. As the concentration of the sample increases anti inflammatory activity increases which concludes (Pk-root+Ag-Np) sample shows good anti inflammatory activity.*

Conclusion: *As the concentration increases the anti inflammatory activity increases. It shows better anti-inflammatory activity than already existing standard drugs. Potential anti-inflammatory drugs can be produced using picrorhiza + silver nanoparticles combination after further research in the future.*

Introduction: *Picrorhiza kurroa is an endangered medicinal herb found in the Himalayas between 3000 and 5000 meters above mean sea level The medicinal properties of P kurroa are attributed to the monoterpenoid picrosides present in the leaves, rhizomes and the roots of the plant However, no genomic information is currently available for P kurroa A total of 710 miRNAs were identified, many of which were found to be associated with molecular responses to temperature changes miRNAs and targets are experimentally validated(Balaji Ganesh, Aravindan, et al., 2025). The availability of the draft genome sequence will facilitate the genetic improvement and conservation of P kurroa The study also provides an efficient way to assemble complex genomes while dealing with duplications when conventional assembly procedures cannot be performed due to duplications.(Sharma et al. 2021)*

INTRODUCTION:

In human history, silver has been used extensively for a very long time. Jewels, utensils, money, and dental alloys are a few of its uses. Among the many uses for silver, its ability to disinfect is being used for sanitary and therapeutic purposes, including the treatment of mental illness, nicotine addiction, and infectious diseases like syphilis and gonorrhea(Gulbranson, Hud and Hansen, 2000).It has been proven that silver nanoparticles have anti-inflammatory, antimicrobial, and antibacterial effects. The method used in our work produces silver nanoparticles quickly and cheaply. With the help of these silver nanoparticles, new information regarding pharmacological uses, such as anti-cancer, larvicidal, medicinal materials, and gadgets, is acquired(Balaji Ganesh, Anees, et al., 2025). As a result, the field of bio nano-medicine will benefit from these biogenetically integrated silver nanoparticles5. Due to their multi-target strategy, silver nanoparticles (AgNPs), which may be produced utilizing physical, chemical, or biological (green synthesis) techniques, have displaced all other metal nanoparticles' unique anti-microbial characteristics (Mulani et al., 2019; Sharma et al., 2021).Other benefits include their many different mechanisms of action, natural antibacterial properties, and greater surface area to volume 14 ratio(Rajchakit and Sarojini, 2017).AgNPs work in a variety of ways, including by damaging cell walls through the electron transport transduction pathway, penetrating as a result of released Ag^+ ions, reacting with intracellular targets, and ultimately destroying structures like cell walls, cell membranes, DNA, proteins, and other cellular components(Qayyum, Oves and Khan, 2017),(Bose and Chatterjee, 2015)).The need for AgNPs is rising because they are great candidates for antibacterial characteristics, therapies, drug delivery agents, etc. The

silver nanoparticles must be biocompatible and chemically free to be used in bioprocesses(Sulaiman et al., 2013).AgNPs should be devoid of harmful compounds that may be formed as a byproduct during their synthesis for the best use of AgNPs in biological applications. To accomplish this, the biosynthesis of AgNPs is advised, in which plant biomolecules are used to create silver nanoparticles by reducing them to the metal state. This technique, often known as "green synthesis," is exceedingly straightforward, precise, and economical((Zhang et al., 2016)).

An early, protective, homeostatic immune response to tissue stress is inflammation. Pro-inflammatory cytokine production and immune system cell activation are its defining features(Eming, Krieg and Davidson, 2007).Because of developments in nanotechnology, nanoparticles with improved absorption and release rates may allow for lower dosages while also enhancing safety thanks to fewer adverse effects(Jain, 2008). The ability of the medicine or carrier to cross physiological barriers like the blood-brain barrier, the branching routes of the pulmonary system, and the constrictive epithelial junctions of skin is another benefit of the nanoscale size(Jain, 2008; Nouailhat, 2010)This plant's roots and rhizomes have historically been used to treat inflammatory and immune problems, liver illnesses, asthma, allergies, and other health issues(Atal et al., 1986).Iridoid compounds from P. kurroa, including kutkin, picroside-1, and kutkoside, have demonstrated inhibitory effect on a variety of phlogistic agents in experimental experiments.A clinical experiment with rheumatoid arthritis patients also showed that P. kurroa extract has a protective effect(Prakash et al., 2020).Nonsteroidal anti-inflammatory medications (NSAIDs) and glucocorticoids, complemented with biological therapy in resistant instances, are the only traditional treatments for inflammatory diseases(Salgotra and Zargar, 2020).Despite the fact that these therapeutic methods are successful in alleviating disease conditions, prolonged and chronic usage of these substances can have substantial negative effects.such as bleeding, stomach ulcers, and the development of opportunistic infections as a result of immunosuppression. According to U.S. epidemiological statistics, gastrointestinal bleeding and stomach ulcers linked to extended NSAID use cause thousands of arthritis sufferers to pass away every year(Umar et al., 2014; Salgotra and Zargar, 2020).

The response of living tissues to stimuli produced by inflammatory agents, such as physical trauma, heat, microbial infections, and noxious chemical irritations, is known as inflammation. Cells' reactions to inflammation can result in pathological symptoms as redness, heat, swelling, and discomfort as well as compromised physiological processes(Palani et al., 2025). Many diseases, such as cancer, arthritis, and stroke, have pathological processes in which inflammation has been involved(Boengler et al., 2008). Protein denaturation has been linked to inflammatory responses that are more common and lead to a variety of inflammatory disorders, including arthritis(Kaminska, 2005). According to Opie, protein denaturation in cells or in ground substance may be the cause of tissue harm over the course of a person's lifetime. Consequently, the ability of a drug to prevent protein denaturation indicates apparent potential for anti-inflammatory activity(Pearson et al., 2001).

AIM: To analyze the anti inflammatory activity of picrorhiza kurroa root mediated synthesized silver nanoparticles.

MATERIALS AND METHODS

Picrorhiza kurroa root extract preparation, silver nanoparticle solution preparation, preparation of (Pk+Ag-Np) solution, centrifugation, (Pk+Ag-Np)pellets collection, Egg albumin assay and bovine serum albumin assay were conducted

Picrorhiza kurroa root is grinded into a fine powder. 4.5 grams of picrorhiza kurroa root powder is taken and added to 150 ml distilled water. Boil the solution in the heating mantle for 10-15 mins at 50 % after boiling the solution nicely for 15 mins. 50 ml of the solution is filtered to get the picrorhiza root extract.

Now add (20 milli moles of silver nitrate)(0.59g) in 50 ml distilled water. The 50 ml of silver nitrate solution is mixed with picrorhiza kurroa root extract in the conical flask. Cover it with aluminum foil and keep it in the shaker and color changes of the solution are observed. At specific time intervals UV readings are taken for the picrorhiza kurroa root mediated silver nanoparticle sample.

After 48 hours 14ml of picrorhiza kurroa root sample is kept for centrifugation at 8000 rpm for 8 mins. The supernatants are discharged and the pellets are collected using a filler.
Anti-inflammatory activity is tested using Egg albumin assay and Bovine serum albumin assay.

BOVINE SERUM ALBUMIN DENATURATION ASSAY

The anti-inflammatory activity for (pk+Ag-Np) was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of Picrorhiza kurroa root mediated synthesized silver nanoparticles solution of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin(1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.
Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

EGG ALBUMIN DENATURATION ASSAY

A 5ml solution was made which consisted of 2.8ml of freshly prepared Picrorhiza kurroa root mediated synthesized silver nanoparticles solution phosphate buffered saline of pH - 6.3, 0.2 ml of egg albumin extracted from hens egg. Specific concentrations were prepared separately for as (10µL,20µL,30µL,40µL,50µL). Diclofenac sodium was used as the positive control.. Then the mixtures were heated in a water bath at 37°C for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

BOVINE SERUM ALBUMIN ASSAY

CONCENTRATION	WAVELENGTH	ABSORBANCE
10µl	660nm	0.336
20µl	660nm	0.274
30µl	660nm	0.234
40µl	660nm	0.196
50µl	660nm	0.08

EGG ALBUMIN DENATURATION ASSAY

CONCENTRATION	WAVELENGTH	ABSORBANCE
10µl	660nm	0.354
20µl	661nm	0.281
30µl	662nm	0.256
40µl	663nm	0.208
50µl	664nm	0.106

FIGURE 1 Picrorhiza kurroa root extract preparation(boiling in heating mantle)

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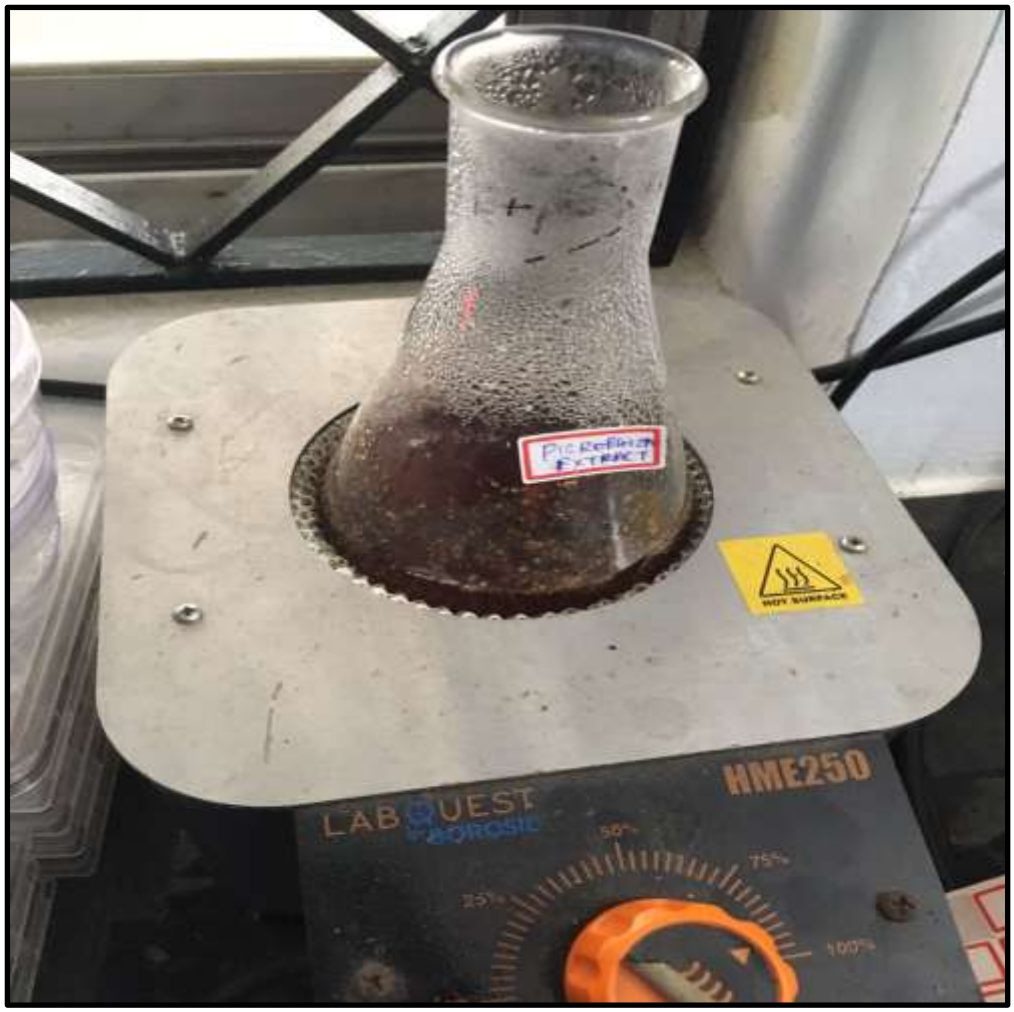

FIGURE 2 Picrorhiza kurroa root extract boiled filtered

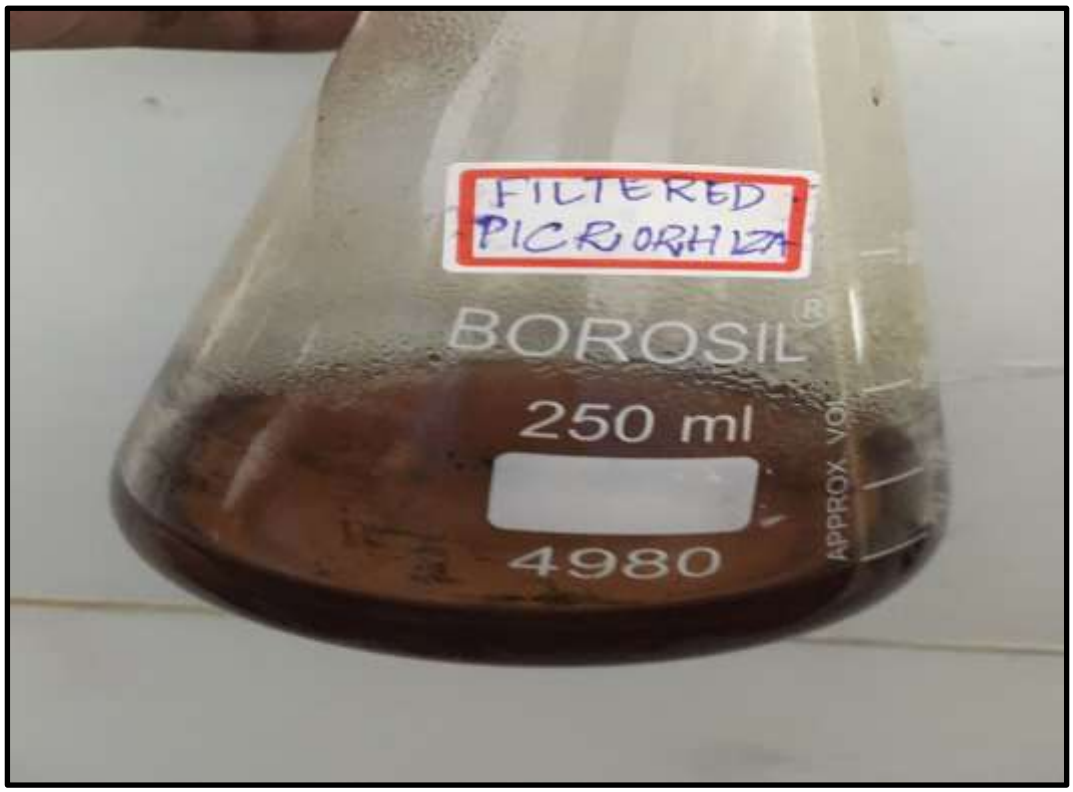

FIGURE 3 Preparation of (Picrorhiza kurroa root + silver nanoparticles) solution

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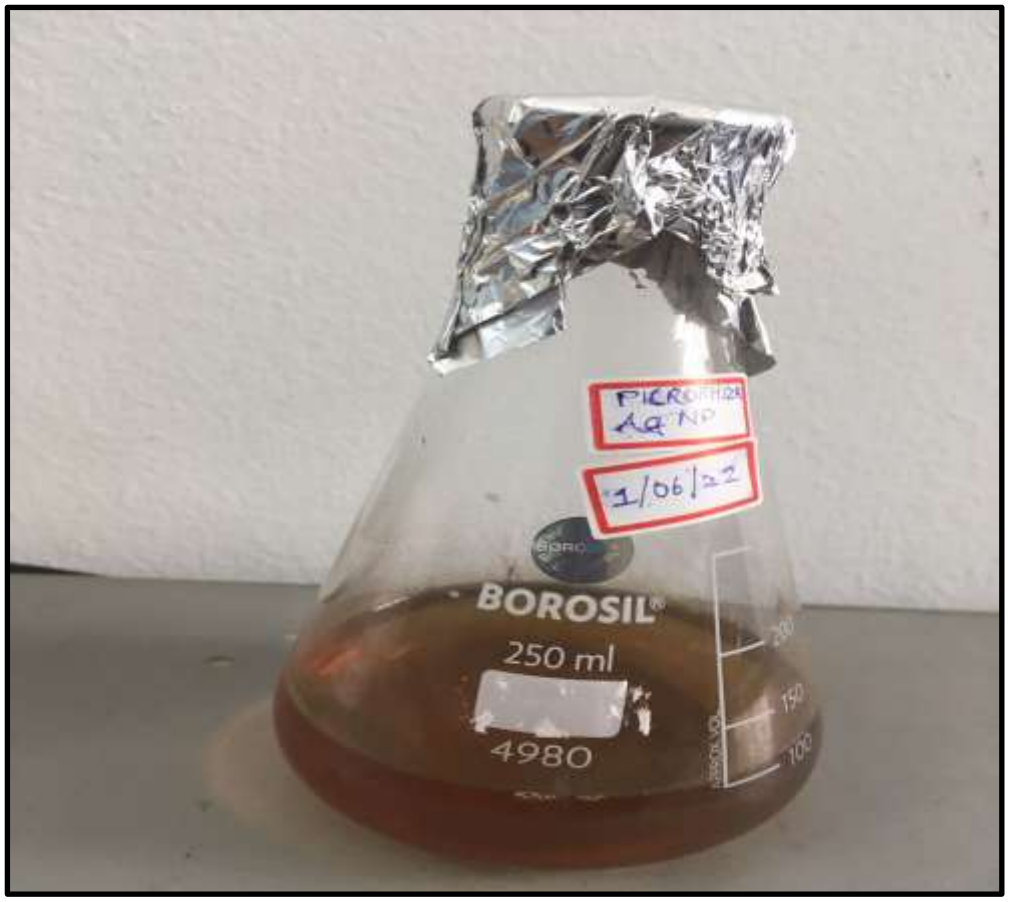
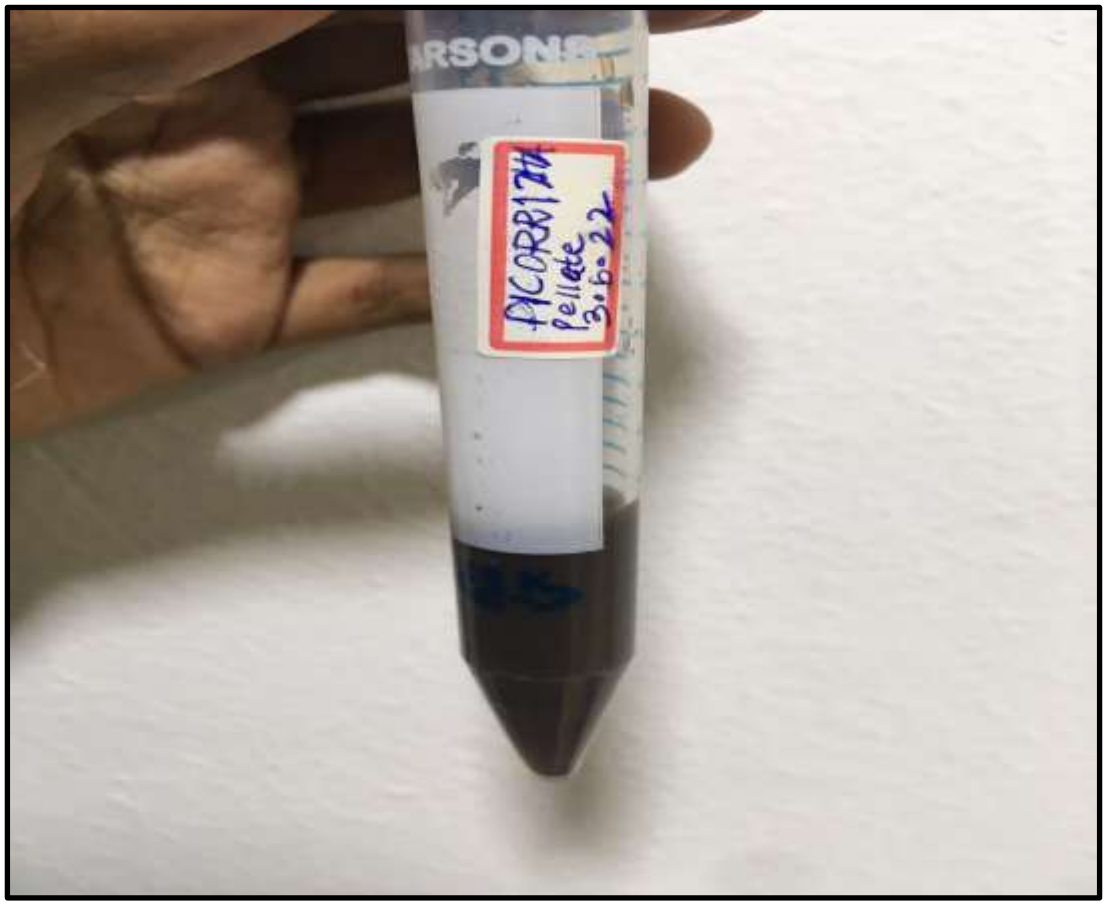


FIGURE 4 Picrorhiza kurroa root+Ag nanoparticles pellets collection (supernatant after centrifugation)



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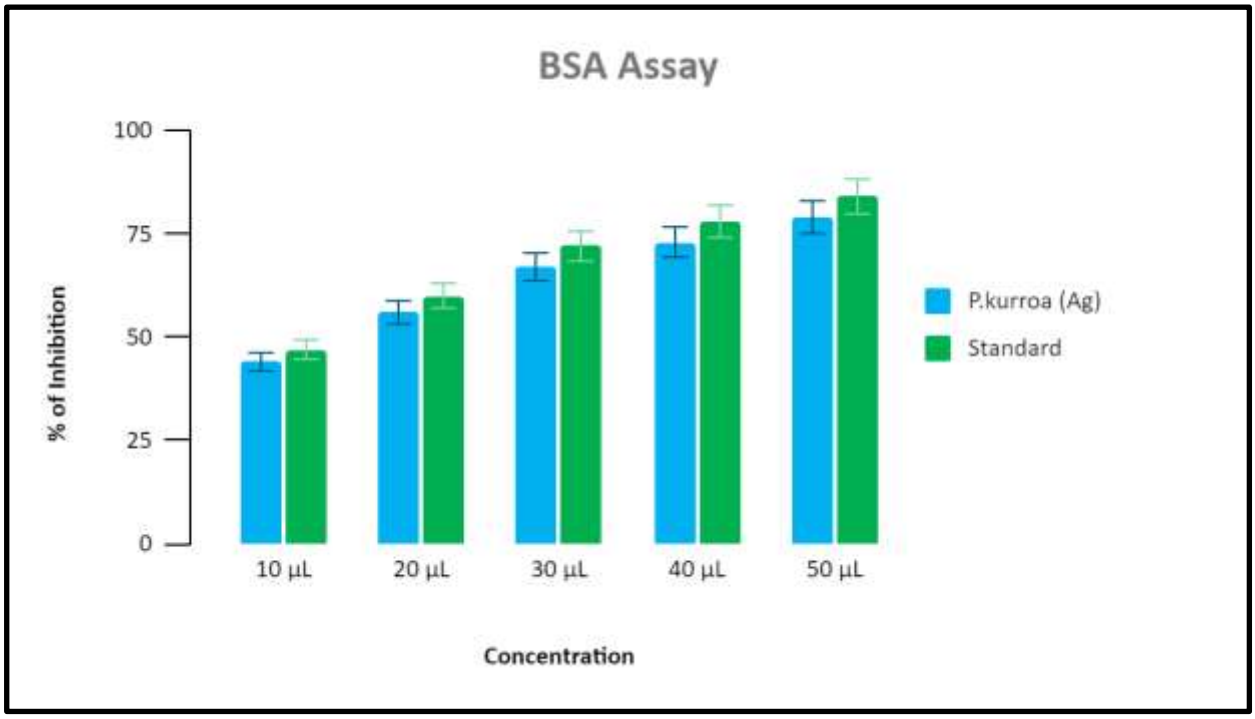
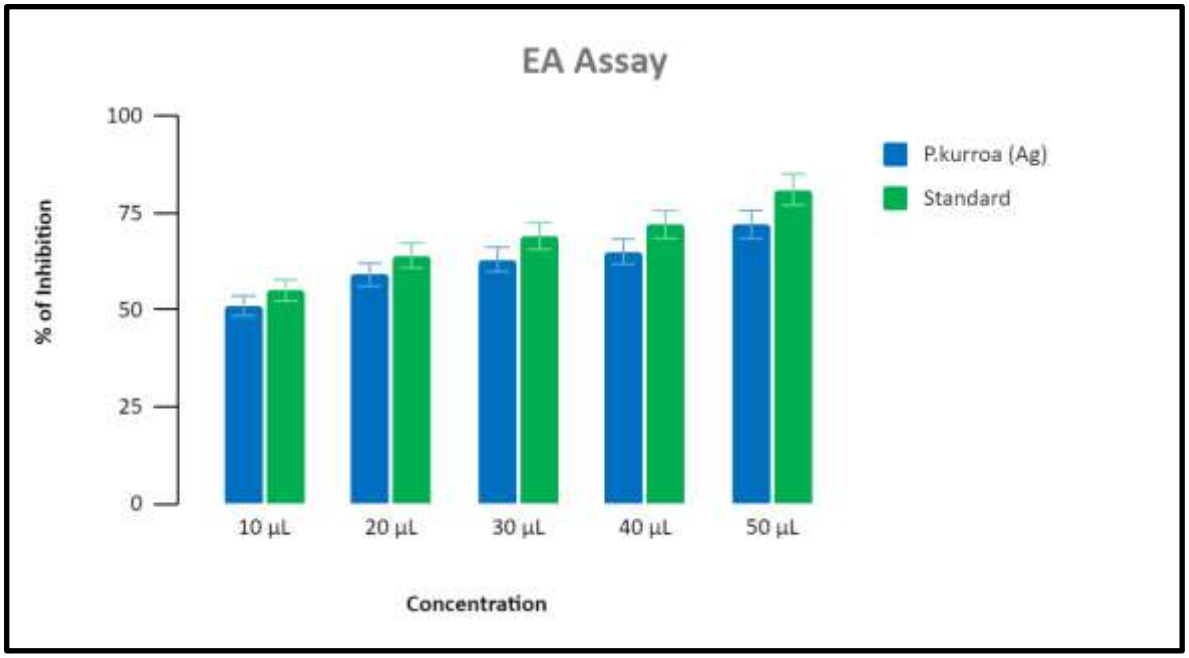
RESULTS

GRAPH 1



X axis represents the concentration of my sample taken at different intervals
Y axis represents the % of inhibition of my sample at different concentrations

GRAPH 2



X axis represents the concentration of my sample taken at different intervals
Y axis represents % of inhibition of my sample at different concentrations

DISCUSSION

BOVINE SERUM ALBUMIN ASSAY

In this assay Bovine serum albumin protein is used and its rate of denaturation is analyzed to find out the samples anti inflammatory activity of the sample(Pk+Ag-NP). Here for comparison already existing standard drug Diclofenac sodium was taken and DMSO was taken as control. As the concentration of the sample increases from 10µl to 50µl the denaturation of the protein bovine serum albumin increases which is directly proportional to the % of inhibition(48% to 80%). Anti inflammatory activity increases as the concentration of the sample increases.

EGG ALBUMIN ASSAY

In this assay egg albumin protein is taken instead of bovine serum albumin protein. Here diclofenac sodium was taken as positive control. As the concentration of the sample increases from 10µl to 50µl the denaturation of the egg albumin protein increases which is directly proportional to the % of inhibition(48% to 80%). Anti inflammatory activity increases as the concentration of the sample increases.

Silver nanoparticles are created using plant extracts from Picrorhiza kurroa. The primary benefit of using plants. It is simple to create silver nanoparticles using extracts. Accessibility, Safe and mostly non-toxic plants, which can lessen the ions of silver. They possess a variety of metabolites that can aid in the speedier synthesis and reduction of silver ions. Herbal compounds such as terpenes, flavones,Aldehydes, amides, carboxylic acids, and ketones are directly engaged in the creation of silver nanoparticles and ion reduction. Additionally, the green synthesis process can create AgNPs at room temperature, is inexpensive, and is environmentally benign(Narchin et al., 2018).A visible transformation of the reaction solution's color from colorless to dark brown during the reduction phase indicates the creation of the AgNPs. Under a Fields Emission Scanning Electron microscope, the produced nanoparticles were seen (FESEM). Similar outcomes for silver nanoparticles produced by plants were also reported(Elumalai et al., 2017).The nanoparticles were discovered to be spherical and of nanoscale size. In the production of AgNPs, Picrorhiza kurroa extracts may function as a capping and reducing agent. AgNPs from the PSA were discovered to have a particle size of 267 nm. The green produced AgNPs' zeta potential was discovered to be a strong peak at 50.6 mV. This shows the created nanoparticles were quite stable('Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity', 2019).

Rats exhibited anti-inflammatory action, according to Bhol and Schechter(Wong et al., 2007). Nanocrystalline silver (NPI 32101) was administered intravenously to rats at a dose of 4 mg/kg or orally at a dose of 40 mg/kg, both of which dramatically reduced colonic inflammation. A dose-dependent fast healing and improved cosmetic appearance were seen in mice treated with AgNPs. AgNPs also showed notable antibacterial qualities, a decrease in wound inflammation, and a regulation of fibrogenic cytokines(Tian et al., 2007). In a follow-up investigation to the prior study, Wong et al. used both in vivo and in vitro models to gather additional evidence for the anti-inflammatory properties of AgNPs. They discovered that AgNPs are able to down-regulate the quantities of inflammatory markers, suggesting that AgNPs could suppress inflammatory events in the preliminary stages of wound healing.According to research using a porcine contact dermatitis model, nanosilver therapy greatly enhances apoptosis in inflammatory cells and lowers levels of proinflammatory cytokines(Nadworny et al., 2010). AgNPs produced biologically can suppress UV-B-induced cytokine production in HaCaT cells as well as edema and cytokine levels in the tissues of the paws(David et al., 2014).

The Ag NPs employed in this study were created utilizing a green synthesis technique that produced AgNPs through chemical reduction and then stabilized them using an amla extract aqueous extract. These AgNPs were discovered to be extremely stable. The resulting solution was reddish orange when aqueous extract of amla was added to a solution of silver nitrate and agitated for 1 hour(Ahmad et al., 2003). The excitation of the metal nanoparticles' surface plasmon vibration is what causes the color to change (to a brownish orange). The SPR bands of the silver colloid occur at various intervals between 428 and 438 nm, and the maximum is red-shifted between 428 and 438 nm. The amla's polyphenols, which are biomolecules that stabilize nanoparticles, were then used to cap the particles. This investigation revealed that Because to agglomeration and an average particle size distribution of 188 nm, the surface of nanoparticles with spherical and cubic forms is difficult. These materials (PE-AgNPs) are produced using a low-cost, environmentally friendly method. Additionally, this technique validates the characterized AgNPs that Drug is carried, and it acts on any equivalents.Using the MTT assay, the cytotoxicity of PE-AgNPs on laryngeal Hep2 cells was examined (viability assay). Various anticancer research have already used the Hep2 cell line(Moalic et al., 2001; Ahmad et al., 2003). According to the results of the MTT experiment, cells exposed to PE-AgNPs (5–60 g/mL) showed reduced mitochondrial activity.

A rat liver derived cell line (BRL 3A) subjected to silver nanoparticles at concentrations in the range of 5 to 50 g/mL showed cytotoxicity due to impaired mitochondrial function, and we discovered AgNPs treatment (24 hours incubation) dramatically decreased percentage of cell viability in Hep2 cells(Hussain et al., 2005).

This may be due to the porin-like beta barrel proteins that are present and the thinner peptidoglycan layer in gram-negative bacteria as opposed to gram-positive bacteria. However, it has also been noted that expanding NPs' surface area likewise increases their surface energy, boosting their efficiency .Therefore, smaller NPs with a higher surface-to-volume ratio exhibit considerable antibacterial action even at a lower concentration. According to a report, Gram-positive bacteria have cell walls made of a thick peptidoglycan layer that forms a rigid structure, making it difficult for the AgNPs to penetrate those walls. Gram-negative bacteria, on the other hand, have cell walls made of a thinner peptidoglycan layer, which may explain why Gram-negative bacteria display a maximum zone of inhibition of 10.75 mm. The silver cations produced by AgNPs functioning as reservoirs of Ag+ (bactericidal agent) ions are undoubtedly responsible for the strong bactericidal activity. It is hypothesized that these NP-derived ions will adhere to the negatively charged bacterial cell wall and break it, causing protein denaturation and cell death (Gopinath et al., 2012; Vanlalveni et al., 2022).

CONCLUSION

As the concentration of the sample increases the anti inflammatory activity increases. It shows better anti-inflammatory activity than already existing standard drugs.It has huge potential to produce anti inflammatory drugs.Potential anti-inflammatory drugs can be produced using (picrorhiza + silver nanoparticles) combination after further research in the future. Even in dental fields drugs can be produced to control inflammation of gums during periodontitis.

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