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Antioxidant activity of silver nanoparticles using Picrorhiza Kurroa root extract.

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ABSTRACT

Introduction: Picrorizha Kurroa, also known as "KUTUKI," is a small perennial herb in the scrophulariaceae family. The rhizome of Picrorizha has traditionally been used to treat worms, constipation, fever, and liver ailments. Iridoid glycoside, picroside LIl,III, and kutikoside are the most important active constituents of Picrorizha. Silver and its compounds, including silver nanoparticles, have antimicrobial properties. According to the findings of the silver nanoparticles study, antibacterial agents inhibit resistance strains through a variety of mechanisms, including actions involving the induction of oxidative stress and interactions with enzymes and proteins. The study's goal is to determine the antioxidant activity of silver nanoparticles synthesised with Picrorhiza Kurroa root extract.

Materials And Methods: Firstly wash the conical flask and 1000 grams of Picrorizha is taken. 100 ml of distilled water was added. Add AgNO3 solution, mark six centrifugation tubes and the extract was filled with 14mleach. Results were analysed using the UV visible spectrometer and assays (DPPH, H2O2).

Results: There was a dose dependent increase in the zone of inhibition of DPPH and H202 assay from CONC. ranging from 10- 50 ug/ml.

Conclusion: This study, shows that the ethanol extracts of Picrorizha exhibits wide range of antioxidant activity against ROS and oxidative damage. The Antioxidant activity was determined using DPPH assay and H202 assay.

Keywords: *Antioxidant, silver nanoparticles, Picrorizha, Assays, Free - radicals*

INTRODUCTION

Picrorhiza kurroa, also known as katuki in Indian, is a small perennial herb in the scrophulariaceae family. Picrorhiza rhizome has traditionally been used to treat worms, constipation, low fever, scorpion sting, asthma, and liver ailments. Antioxidants and their anti-diabetic effects are the focus of current research. The molecules that inhibit the oxidation of other molecules necessitate the study of antioxidant importance. As an oxidising agent, oxidation is a chemical reaction that transfers electrons from a hydrogen substance. Free radicals are produced during oxidation reactions. Radicals, in turn, set off chain reactions. A chain reaction can occur in a cell, causing damage or death.[1] (Subedi 2006)

In diabetes, the combination increases oxidative stress and decreases anti-oxidant status, resulting in greater vulnerability to the damaging effects of free radicals.[2](Sies 2020)

Iridoid glycoside picrosides I, II, III, and kutkoside, collectively known as kutkin, are the most important active constituents of Picrorhiza. Many other active constituents, such as nine cucurbitacin glycosides, apocynin, and drosin, have been identified.[3](Pai and K. 2021) The roots of P. kurroa yielded kutchina-glucoside bitter principle after extraction with petroleum ether. Finally, a stable mixture of two glycosides, picroside-1 (6''O-cinnamoyl catalpol) and a new glucoside kutkoside, was reported, which was characterised by 10-O-D-mannitol, kuttikol, kutki sterol, and a ketone that was found to be identical to apocynin isolated from Apocynum cannabinum. It was reported that apocynic homologs were synthesised.[4] (Sanjay and Shukla 2021)

Certain foods contain antioxidants, which may help to prevent some of the damage caused by free radicals by neutralising them.[5](Ames et al. 1993)These include antioxidants, vitamins A, C, and E, as well as the minerals copper, zinc, and selenium. Other dietary food compounds, phytochemicals found in plants, were thought to have greater antioxidant properties than vitamins or minerals.[6](Shirwaikar et al. 2003)Non-nutrient antioxidants, which include phytochemicals, are referred to as such (such as lycopenes in tomatoes and anthocyanins found in cranberries). An antioxidant is a chemical that prevents other chemicals from oxidising.[7](Patil et al. 2005) They protect key cell components by neutralising the damaging effects of free radicals, which are formed during the cell metabolism from natural byproducts. 1,2 Free radicals are formed when oxygen is metabolised or formed in the body by chemical species that have an unpaired electron in the molecule's outer (valance) shell.[8,9](Evans et al. 2016)(Devasagayam and Kesavan 1996. This is why free radicals, which are highly reactive and can react with proteins, lipids, carbohydrates, and DNA, exist.[10,11](Badarinath 1986)(Rai et al. 2009)resistant

Antimicrobial properties of typical silver and its compounds including silver nanoparticles (AgNPs) .[12,13](Pandit 1970)(Abbasi et al. 2016) In light of the fact that AgNPs have been studied as antibacterial agents that inhibit strains, the multiple mechanisms of action, which include induction of oxidative stress, inhibition of DNA replication, and interactions with enzymes and proteins. [14,15](Jiang et al. 2008)(Hwang et al. 2012)

Silver nanoparticles, on the other hand, have the potential to be used in the treatment of cancer or degenerative Alzheimer's disease due to their antioxidant properties.[16,17](Mittal et al. 2012)(Bedlovičová et al. 2020)

Our team has extensive knowledge and research experience that has translate into high quality publications[20 - 28] (Neelakantan et al. 2013; Aldhuwayhi et al. 2021; Sheriff et al. 2018; Markov et al. 2021; Jayaraj et al. 2015; Paramasivam et al. 2020; Li et al. 2020; Gan et al. 2019; Dua et al. 2019; Mohan and Jagannathan 2014)

The aim of the study is to check the antioxidant activity of silver nanoparticles synthesised using Picrorizha kurroa root extract.

MATERIALS AND METHODS

First wash the two conical flasks and funnel without any previous left over. Take 1000 gms of Picrorhiza on a conical flask. Add 100 ml of distilled water. Stir it well until it gets mixed. Place it in the heating mantle for 10 to 15 mins in a temperature of 60-70 degree celsius. Wait until it gets heated. It takes 10 mins to get heated. 70 ml of distilled water is taken on a measuring flask after the preparation of silver nitrate. Mix distilled water and silver nitrate. Mix it well. Now take another conical flask, place the funnel over it. Keep the cotton cloth over the funnel for filtration. Now pour the Picrorhiza solution which was kept in another flask previously. It gets filtered and collects the extract in the conical flask. Now take the measuring scale pour 30 ml of filtered Picrorhiza Extract and add it to the silver nitrate solution.Mark it as Picrorhiza silver Nitrate nanoparticles . Cover it with aluminium foil and place it in an orbital shaker . Ag - argentum - silver - 1 to 2 days colour changes have to be noted.

FIG: 1 Picrorizha Kurroa sample.

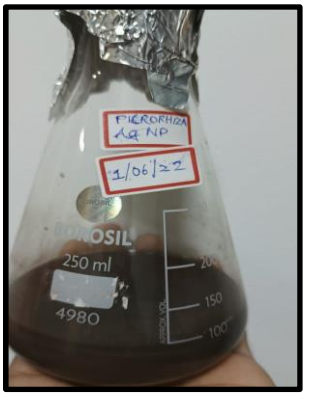


FIG: 2 Picrorizha root extract

Antioxidant Activity

Dpph Method

DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of Picrorizha kurroa extract interceded silver nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Ascorbic acid was used as standard. The percentage of inhibition was determined from the following equation,

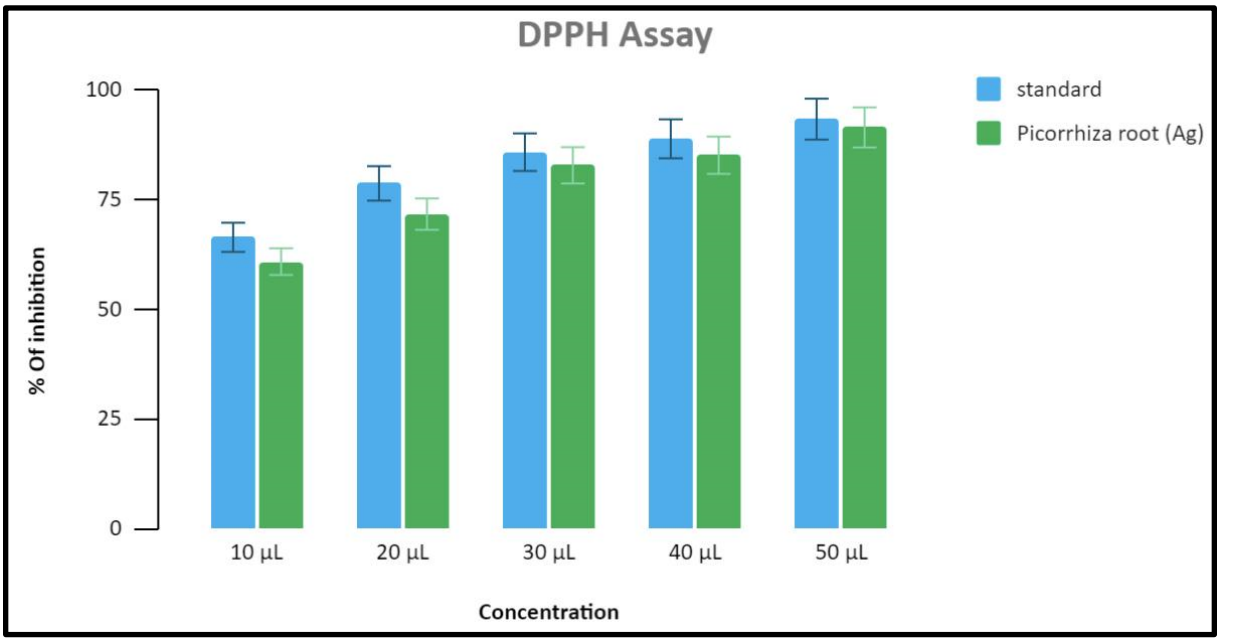
% inhibition= Absorbance of control- Absorbance of test sample × 100

Absorbance of control

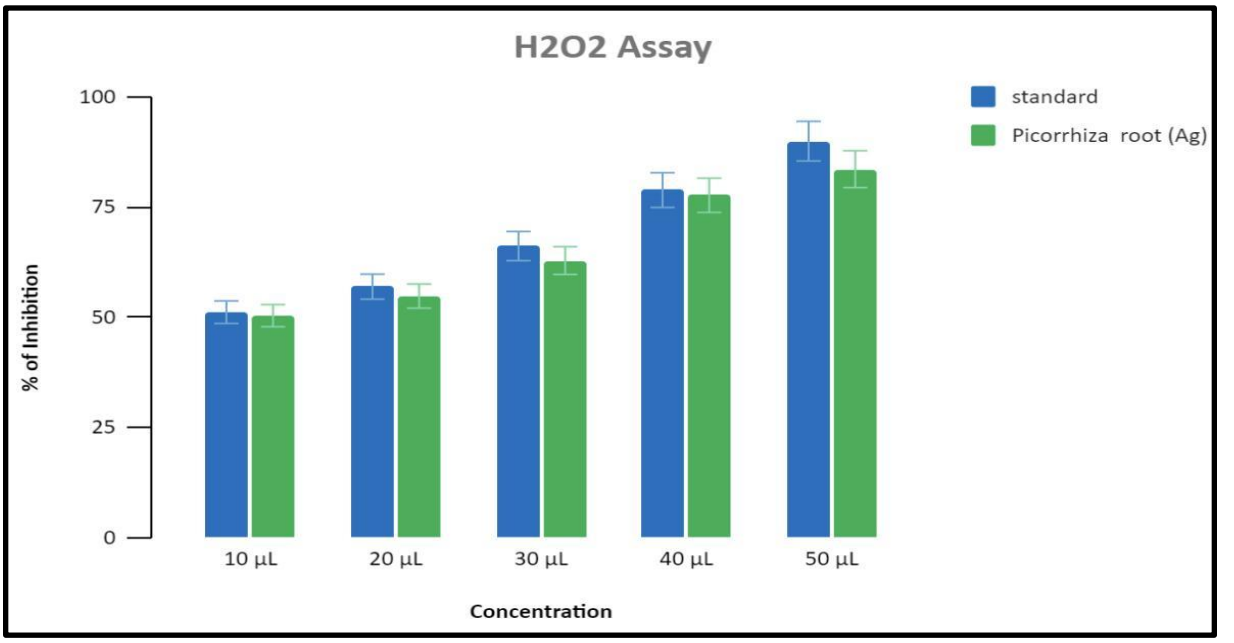
Hydroxyl Radical Scavenging Assay

All solutions were prepared freshly.1.0mL of the reaction mixture contained 100µL of 28mM of 2-deoxy-2-ribose (dissolved in phosphate buffer,pH 7.4), 500µL solution of various concentrations of thePicrorizha kurroa (10µL,20µL,30µL,40µL,50µL) 200µL of 200µM Fecl3 and 1.04mM EDTA (1:1 v/v),100µL H2O2(1.0mM) and 100µL ascorbic acid(1.0mM).After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532nm against the blank solution . Vitamin E was used as a positive control.

RESULT



GRAPH 1 represents the DPPH assay. X axis represents concentration of the extract on microlitre.Y axis represents the percentage of inhibition. There was a dose dependent increase in the percentage of inhibition in the concentration ranging from 10 ug to 50 ug per ml.



GRAPH 2 represents the H2O2 assay. X axis represents concentration of the extract on microlitre.Y axis represents the percentage of inhibition. Increase in the zone of inhibition from concentration 10ul to 50ul. At 40ul and 10ul standard value and extract result shows equal rate of percentage of inhibition.

DISCUSSION

The study shows that the ethanol extracts of Picrorhiza kurroa exhibited a wide range of antioxidant activity against different reacting oxygen species(ROS) . The antioxidant activity was determined using DPPH and H2O2 assay.

The use of various in vitro methods for the basic study of antioxidant activity is a suitable choice, either in mechanism of action such as hydrogen transfer or electron transfer. The mechanism of antioxidant action of silver nanoparticles - attributed to the fact that silver can exist in two oxidation states (Ag+ and Ag2+) depending on the reaction conditions, the produced AgNPs was able to quench free radicals by donating or accepting electrons - was studied using these methods. In vivo activity-based methods have revealed that nanoparticles have a more complicated mechanism of action in biological systems.[17](Bedlovičová et al. 2020)

Antioxidants' protective effect is still being studied all over the world. For example, men who consumed a lot of the antioxidant lycopene (found in tomatoes) were less likely to develop prostate cancer than other men. Lutein, spinach, and corn, which have been linked to a lower incidence of eye lens degeneration, are linked to vision loss in the elderly. Flavonoids (such as the tea catechins found in green tea) were thought to contribute to Japan's low rate of heart disease.[2](Bedlovičová et al. 2020; Sies 2020)

Using this simple technique, the antioxidant capacity of non-refined seed oils was compared to that of refined oils. The method used to measure or calculate antioxidant activity has a significant impact on the results because oxidation reactions in the complex occur both in foods and in vitro. The liberation of antioxidants during food digestion may differ from the liberation of the same during antioxidant test extraction.[10](Badarinath and Subrahmanyam 1986)

The DPPH method measures the stable free radical virtue of delocalization of spare electrons across the molecule as a whole, preventing dimerization that would occur in most other free radicals.

AgNPs have a yellowish brown to dark brown colour due to Surface Plasmon Resonance, depending on their size and shape. The colour change in the reaction solution was used to perform preliminary identification of the nanoparticle formation. When an AgNPs colloidal solution was subjected to UV-Visible spectral analysis, sharp bands of silver colloids were observed at 400-450 nm, which had previously been reported by other researchers. Two control reactions were kept in aqueous AgNO3 solutions with no colour; the plant extract solution is pale yellow.[16](Mittal et al. 2012)

LIMITATIONS

Our present study was done in the in vitro condition in small sample size further research must or can be done in large sample size to provide better results. Much more assays need to be checked for the antioxidant activity.

CONCLUSION

The present study enlightens the antioxidant activity of Picrorhiza kurroa root extract using silver nanoparticles which was determined using DPPH assay and H2O2 scavenging assay.

FUTURE SCOPE

Our present study was done in invitro condition of antioxidant activity of Picrorhiza root extract using silver nanoparticles. Further research targeting animal models in vivo conditions that would substantially add antioxidant properties to the nanoparticles and it would be a better drug of choice.

CONFLICT OF INTEREST

There is no conflict of interest.

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AUTHOR CONTRIBUTION

Mr. Shanmugam S B - Literature search, data collection, manuscript writing.

Dr.Rajesh Kumar Shanmugam-contributed in designing the study,execution of the project,statistical analysis,manuscript drafting.

Mrs.S.Sangeetha-contributed in study design,guiding the research work,manuscript drafting.manuscript correction.

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