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Saif Alam
Assistant Professor, Department of Pharmacy, DRON of Pharmacy, Meerut, Uttar Pradesh, India

Wasim Akhtar
Assistant Professor, Department of Pharmacy, RIT College of Pharmacy, Meerut, Uttar Pradesh, India

Manish K Prajapati
Assistant Professor, Department of Pharmacy, RIT College of Pharmacy, Meerut, Uttar Pradesh, India

Naziya
Assistant Professor, Department of Pharmacy, RIT College of Pharmacy, Meerut, Uttar Pradesh, India

Dr. Juli Tansariya
Associate Professor, Department of Botany, RIT College of Pharmacy, Meerut, Uttar Pradesh, India

Tahreej Chaudhary
Assistant Professor, Department of Pharmacy, RIT College of Pharmacy, Meerut, Uttar Pradesh, India

Atul Pratap Singh
Assistant Professor, Department of Pharmacy, SOPS, IIMT University, Meerut, Uttar Pradesh, India

Corresponding Author:
Saif Alam
Assistant Professor, Department of Pharmacy, DRON of Pharmacy, Meerut, Uttar Pradesh, India

Pharmacological properties of *Rhamnus virgata*: A comprehensive review

Saif Alam, Wasim Akhtar, Manish K Prajapati, Naziya, Dr. Juli Tansariya, Tahreej Chaudhary and Atul Pratap Singh

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Abstract
Physcion, kaempferol, emodin, quercetin, rhamnocitrin, maesopsin, and herbacetin are the main chemicals found in *Rhamnus virgata*, which is a huge shrub/small tree belonging to the Rhamnaceae family and It is widespread in India, Pakistan (Swat, Kashmir) and China. Strong therapeutic potential in the treatment of parasitic infections, spleen diseases and leg swelling been demonstrated for *Rhamnus virgata*. These therapeutic potentials include antimicrobial, antioxidant, laxative, purgative, and emetic effects. *Rhamnus virgata* leaves extract has been used to synthesise eco- and bio-friendly Ag2ONPs via green chemistry procedures due to the existence of various green chemicals and their therapeutic potentials. *Rhamnus virgata* contains a lot of flavonoids, anthraquinones, and alkaloids, and can serve as anbest option for converting of iron salt to nanoiron. *Rhamnus virgata* leaves extract's medicinal potential, phytochemistry, and Their biological activity necessitates their use in the preparation of ZnONPs and they could be an excellent candidate for the reduction of zinc salts to nanozinc.A comprehensive discussion has been had over the circumstances of the reactions that lead to the synthesis.

Keywords: *Rhamnus virgata*, anthraquinones, rhamnocitrin and kaempferol

Introduction
Rhamnus virgata, a large shrub or small tree with sharp spines and a member of the Rhamnaceae family, is extensively found in India, Pakistan (Swat, Kashmir), and China. *Rhamnus virgata* contains physcion, kaempferol, emodin, rhamnocitrin, herbacetin, quercetin, and maesopsin as its primary chemical constituents [1, 2]. Strong therapeutic potentials for the treatment of parasite contaminations, spleen affliction, and leg swelling have been demonstrated for *Rhamnus virgata*. These therapeutic potentials include antioxidant, laxative, antibacterial, emetic, and purgative actions [3, 4, 5]. It can also serve as well option in the decrease of iron salt to nanoiron. Since, it contains a lot of flavonoids, anthraquinones, and alkaloids. *Rhamnus virgata* leaf extract has been used to create biodegradable and environmentally friendly Ag2ONPs utilizing green chemistry techniques due to the presence of many green compounds and their medicinal potential [6]. *Rhamnus virgata* leaves extract's medicinal potential, phytochemistry, and biological activities made their usage in the production of ZnONPs necessary, and they may serve as an excellent option in conversion of zinc salt to nano-zinc [7].

Taxonomic hierarchy
The following is the taxonomic hierarchy of *Rhamnus virgata*:
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Rosales
Family: Rhamnaceae
Genus: Rhamnus
Species: virgata [8]

Description

Shrubs or trees, dioeciously, near 6 m high and spinose. Younger branches are heavily hirsute; older branches are lustrous, purple-red or red-brown, glabrous, smooth, and end in spines. Branchlets are opposite or subopposite. Adaxially canaliculated, thickly hirsute or puberulent, 4-10-15 mm long petiole; leaf sharp edge splendid green, becoming red when dry; Leaves alternate or opposite, or in clusters on short stalks. Ovate-elliptic, obovate-lanceolate or elliptic, 2.5–8–1.5–3 cm, chartaceous or finely chartaceous, hairy axially above veins or only in corners of veins, semismooth or densely hairy, axially hairy or hairy or semi-bare on veins only, lateral veins 4 or 5 pairs, distinct reticulated veins, 4-merose, flowers unisexual. Petals 3 to 4 mm long, slightly hairy or globous. The flowers are 4-fold and unisexual. Flakes are present and measure 3 to 4 mm. Few, fascicled, and with rudimentary stamens, female flowers have a 2-cleft style. Drupe blue-black, subglobose, 4-5 mm in diameter, with 2 stones; fruiting pedicel 2-5 mm; persisting calyx tube at the base. Abaxially, the margined furrow on the reddish-brown seeds spans around 2/3 to 3/4 of their length. Feb.-June, blooming April-May.

A much branched almost glabrous, deciduous shrub or small tree, usually branches ending in stout sharp thorns. Leaves opposite to sub-opposite, variable in size and shape, elliptic-lanceolate obovate, 2-10 cm x 1-6 cm, membranous, slightly pubescent when young, serrate-crenate, acuminate, cuneate, lateral nerves curved or converging, Petiole 5-6 mm long, slightly hairy, flowers in the leaf axils, 4-merous, unisexual, pedicels 5-8 mm long wiry. Flowers 4-5 mm long, calyx c. 4 mm long, lobes c. 2.5 mm long, lanceolate, acute, lengthier than the hypanthium, c. 2 mm long. Petals spathulate c. 1.5 mm long. Disc glabrous, thin. Fruit obovoid c. 7 mm long 2-4 seeded, seeds shining grooved on the back, ovoid, dark brown. C. 5 mm x 2 mm [9].

Fig 1: *Rhamnus virgata* plant

Fig 2: *Rhamnus virgata* flower

Fig 3: *Rhamnus virgata* leaves

Fig 4: *Rhamnus virgata* bud

Pharmacological activities

Antileishmanial assay

By performing MTT cell viability assays, the cytotoxicity of biogenic IONPs in contrast to *Leishmania tropica* strain KMH23 (amastigote and promastigote cultures) was investigated [10]. To cultivate Leishmania parasites, MI99 medium plus 10%. Fetal bovine serum (FBS) was used. A culture of *Leishmania tropica* with a density of 1 x 106 cells per milliliter was used for the test. The anti-Leishman efficacy of IONP was tested at final concentrations ranging from 1 to 200 g/mL, Amphotericin B and the positive and negative controls, respectively, were DMSO. The 96-well plate containing the NPs to be tested was kept in a 5% CO 2 incubator at 24 °C for 72 hours. The spectrophotometer measurements were made at 540 nm. All treatment surviving promastigotes and amastigotes were counted using an inverted microscope and IC50 values were calculated using GraphPad software [11].

Antileishmanial activity

The 'KMH23 strain' of *L. tropica* causes the neglected tropical illness Leishmania [12]. The parasites are widespread to 100 different countries, with an annual occurrence rate of 1.2 million. Historically used antileishmanial medications are frequently poisonous, ineffective, and very costly. Antimonials were formerly developed as a viable treatment option for leishmaniasis, however, their efficacy has been compromised by *L. tropica* resistance. As a result, the scientific community is working to create various methods of therapy. Since then, much research has been conducted to create MNPs for the treatment of leishmania. Various *in vitro* studies use various MNPs to test the cytotoxicity against leishmanial parasites [13, 14]. However, little research

has been done on the cytotoxicity of biogenic IONPs in contrast to *L. tropica* (KMH23) [11].

As displayed in Figure 5, the antileishmanial action (ALA) of biogenic IONPs was assessed in the current investigation against *L. tropica* (KMH23). Leishmanial parasites were managed with IONPs at various doses (1-200 g/ml) for 72 hours in this study, and *L. tropica* demonstrated dose-dependent inhibition. Because of the rise in IONP concentration, the antileishmanial potential got up. The IC50 values for IONPs against *L. tropica* promastigotes have demonstrated promising antileishmanial potential (IC50: 8.08 g/ml). Similar to how ALA was demonstrated to be effective against *L. tropica* amastigotes from IONP (IC50: 20.82 g/mL), this result is consistent with previous studies using IONP biogenic nanoparticles [15, 16]. The dose-dependent and lower IC50 values of IONPs indicated that they can be used to deliver potent anti-Leishmania drugs in future pharmaceutical products.

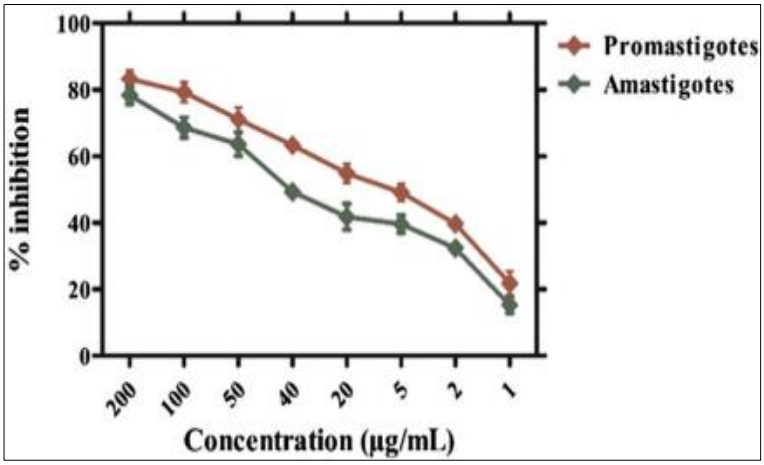

Fig 5: Tests for the cytotoxicity of IONPs Toxic to *Leishmania tropica* cytoplasm.

Anticancer activity

A formerly superior methodology was utilized to test the cytotoxicity capacity of IONPs against the HepG2 (hepatocellular carcinoma) malignant growth cell line [17]. On DMEM medium enhanced with 10% FBS, 1% streptomycin, and 1% penicillin, cells were refined. The HepG2 cells were grown in an incubator at 37°C in humified 5 percent CO2. The MTT cytotoxicity assay was detected in a 96-well microtiter plate by incubating IONPs at various test doses (500-3.9 g/mL) for 48 hours. Each well was filled with MTT solution (20 µl) which was then incubated for three hours. After 100µl of DMSO was added to the culture medium, an additional 20 minutes of incubation ensued. A plate reader with a wavelength of 570 nm was used to measure the amount of formazan produced by the remaining cells. IC50 values were recorded using spreadsheet software [11].

Cancer is a fatal illness that continues to be a main cause of death in both industrialised and rising nations. Its prevalence is steadily rising and is expected to reach 21 million cases by 2030 [18, 19, 20]. Hepatocellular carcinoma, which now ranks as the sixth maximum prevalent disease overall and the second-deadliest cancer for males, and the sixth-deadliest cancer for women, has been linked to 745 517 fatalities. HBV and HCV viral infections, excessive alcohol intake, and toxin exposure (aflatoxin) are risk factors for liver cancer [21]. The HepG2 cancer cell line was used to test the IONPs' anticancer potential. Cancer cells were dose-dependently inhibited after being exposed to different convergences of IONPs (500-3.9 µg/ml) for 24 hours. Our findings demonstrated that IONPs have potent antitumor action. The greatest recorded dose-dependent inhibition, with an 86% death rate, was 500 µg/ml, whereas the anticancer effect declined as the dose level decreased. The determined IC50 value was 13.47 µg/ml. Additionally, our study is consistent with earlier studies on biogenic IONPs done [16]. Figure 6 presents the outcomes.

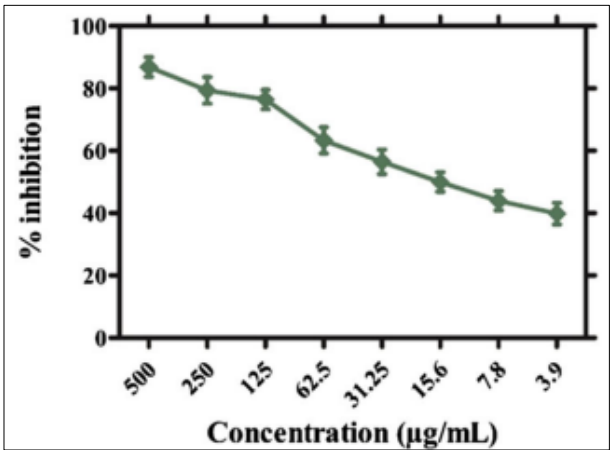

Fig 6: Assays for iron oxide nanoparticles (IONPs') cytotoxicity. HepG2 cell line cytotoxicity.

Antibacterial activity

Antibacterial activity of biogenic IONPs against various bacteria strains that were available was evaluated using the disc diffusion technique. By inoculating the solid cultures on nutrient agar medium, the cultures were restored. The cultures were added to the nutritional broth medium prior to the activity and Stirred in an incubator at 37 °C and 200 rpm for 24 hours. Standardization of microbial cultures was performed by maintaining the OD at 0.5 or 1108 cfu/mL, The filter discs (6 mm) were dried and delicately put over bacterial lawns using sterilized cotton swabs. A positive control disc containing 10 µg of oxytetracycline was employed as a standard. The microbes plates were hatched for 24 hours at 37°C, and the zones of restraint and corresponding MIC values for the varied concentrations (1000-31.25 g/ml) were computed [11].

For bacterial diseases, traditional therapies frequently provide the possibility of antibiotic therapy, However, antibiotic resistance is one of the issues with the drug.

Leading scientists are effectively exploring new ways to combat antibiotic resistance and reduce the likelihood of contracting and spreading these deadly diseases [22, 23]. New nanotechnology methods that look promising will be available for the design and production of novel compounds with distinct antibacterial properties [24].

The effectiveness of biogenic IONPs against different bacterial strains at different concentrations (1000–31.25 g/ml) is shown in Figure 7. Bacillus subtilis (ATCC 6633) and Staphylococcus aureus (ATCC 25923) were gram positive strains, while the gram positive strains were negative. These include Klebsiella pneumoniae (ATCC 4617), Escherichia coli (ATCC 15224), and Pseudomonas aeruginosa (ATCC 9721Most of the bacteria tested were sensitive to IONP. Interestingly, previous studies at very high doses (50 mg/mL) have not shown any chemically mediated effect on P. aeruginosa IONPs. [25] However, the bacterial strains that had the greatest impact on biogenic IONPs were E.coli (CMI: 31, 25 µg/ml) I B! subtilis (MIC: 31.25 µg/ml). A value of MIC of 125 g/ml, P.aeruginosa and Klebsiella pneumonia were found to be minimally susceptible strains. Table 1 contains the MIC values; the positive control was 10 g of pure oxytetracycline. No force has been shown to be more effective than the drug oxytetracycline. Overall, consistent with previous studies, we found likely biogenic antibacterial properties of IONPs. [15, 16, 26] The bioactive functional groups attached to NPs could be the reason for the strong antibacterial activity of NPs. Furthermore, our studies showed that *Rhamnus virgata*-mediated IONP concentration increased with antibacterial capacity [11].

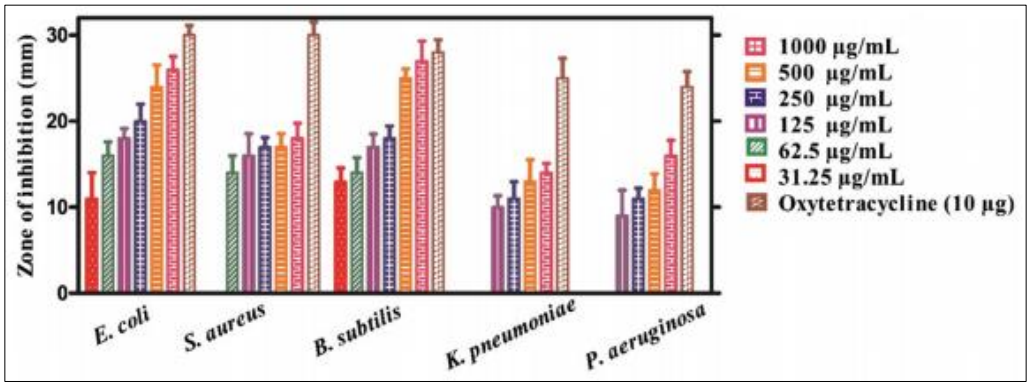

Fig 7: Antimicrobial potential of iron oxide nanoparticles (IONPs). The antibacterial potential of IONP lighting.

Antifungal assay

The disk diffusion technique was used to assess the antifungal potential of IONP. In a shaking incubator with Sabouraud dextrose liquid medium (Oxoid CM0147), fungal spores were cultured in tubes and shaken at 37 °C for 24 hours. The optical density of the liquid culture was set to be 0.5. The progress of fungal straining in sterile Petri plates required the preparation and autoclaving of SDA solid medium. Filter discs were placed gently on media plates after being impregnated with 10 l of sample solution. DMSO discs and amphotericin B (10 g) were used as positive and negative controls, respectively. Moreover, the zone of hindrance on growth plates was estimated in mm following 48 hours of brooding at 28 °C. At various dosages, IONPs with concentrations ranging from 1000 to 31.25 g/ml were utilized. The low concentration of IONP used to calculate MIC values [11].

Antifungal activity

Through Amphotericin B serving as a positive control, the antifungal ability of *Rhamnus virgata*-mediated IONPs was examined against several fungus strains at various doses (1000-31.25 µg/ml). Aspergillus niger (FCBP 0918), Mucor racemosus (FCBP 0300), Candida albicans (FCBP 478), Aspergillus flavus (FCBP 0064), and The various strains of fusarium solani (FCBP 0291) used in the study. While just a small amount of study has been done on IONPs' possible antifungal properties, their antibacterial potential has received significant attention [11].

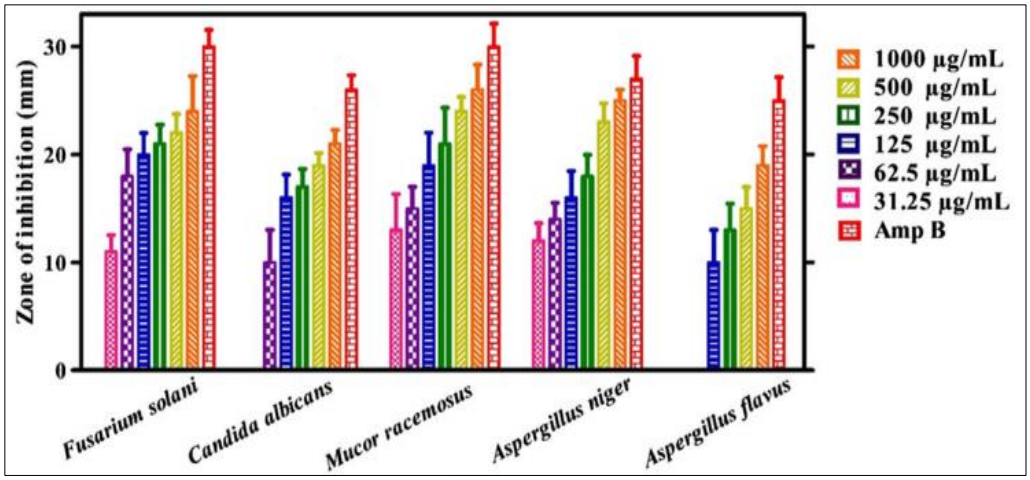

Fig 8: Potential of IONPs to fight bacteria. Antifungal potential of IONPs.

The antifungal action of IONPs got from *Rhamnus virgata* is being educated for the underlying time in the current examination. The antifungal activity of IONPs was evaluated using a variety of fungal strains, including A. flavus, M. racemosus, C. albicans, F. solanito, and A. niger, at concentrations ranging from 1000 to 31.25 g/ml. There was a strong correlation between the test sample's IONP content and our findings. M. racemosus, F. solani, and A. niger were the strains with the highest MIC upsides-31.25 g/ml-while Aspergillus flavus was the strain with the lowest MIC upsides-125 g/ml. However, Amphotericin B exhibited the highest inhibition percentage of any of the test samples [11].

All concentrations of the tested strains suppressed F. solani, A. niger, and M. racemosus. It was discovered that A. flavus was not sensitive to low test concentrations. According to previous work, IONPs' disruption of fungal hypea and spores results in the restriction of fungal development in addition to their ability to produce ROS [27]. In accordance with the current discoveries, earlier studies [15] proved significant dose-dependent antifungal activity. We also deduced that IONPs had a concentration-dependent reaction. Table 2 displays MIC values for several fungi strains [11].

Antioxidant capacities

To test the new trapping potential, a spectrophotometric method was used by adding 2.4 mg of DPPH as a radical to 25 mL of methanol. Various concentrations of IONPs (ranging from 1 to 200 g/ml) were tested for free radical scavenging [17]. DMSO and ascorbic acid (AA) were used as negative and positive controls, respectively. The 200 µl reaction mixture consisted of 180 µl of the reagent solution and 20 µl of the test sample after incubation in the dark for 30 minutes, the reaction mixture was measured at 517 nm on a microplate reader to calculate the amount of DPPH removed by half using the following formula:

$$\%\text{DPPH scavenging} = 1 - \left[\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

The total reducing power (TRP) of biogenic IONPs was tested using the potassium ferricyanide method previously described [28]. AA and DMSO were used as positive and negative controls, respectively. A microplate reader was used to measure the absorbance intensity at 630 nm. The reducing power was calculated in milligrams of equivalent AA. The phosphomolybdenum technique was used to test total antioxidant capacity [29]. The absorbance at 695 nm was measured with an optical microplate reader. Results are expressed in mg AA equivalent per µg of test material [11].

Antioxidant activities

The antioxidant capabilities of IONPs (DPPH, TAC, and TRP free radical scavenging) are depicted in Figure 9. The antioxidant assays were performed in the range of 200–1 mg/ml. In terms of AA equivalents per mg, IONPs had the highest total antioxidant concentration, 51.4 percent, at 200 g/ml. TAC demonstrates the tested chemicals' capacity to scavenge ROS species. *Rhamnus virgata* leaves extracted in aqueous form were employed in the current investigation as a capping, oxidizing, and reducing agent. ROS, which are also associated with IONPs, can be scavenged by a variety of phenolic compounds. To learn more about the presence of the types of antioxidants bound to IONPs, the TRP was examined. This method was used to study reductones, which have antioxidant potential with H atoms and can damage free radical chains [30].

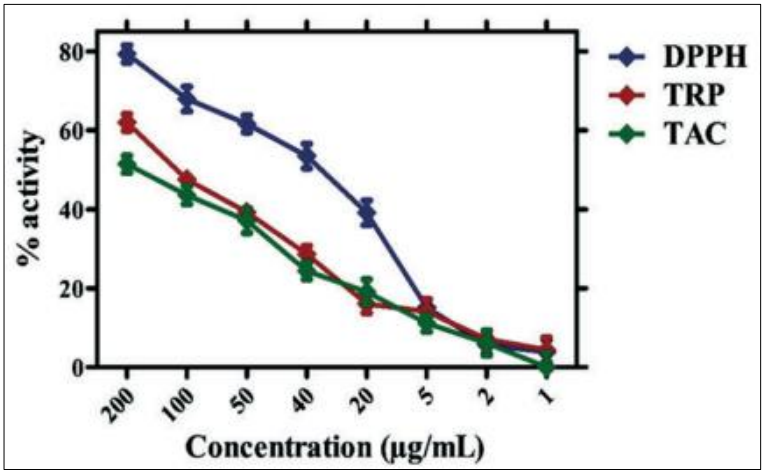

Fig 9: Iron oxide nanoparticles (IONPs) biocompatibility, antioxidant, and enzyme inhibition assay. Different antioxidant activities of IONPs.

Biogenic IONPs have demonstrated significant antioxidant activity. Due to the decrease in IONP concentration, the reducing power decreases. At 200 µg/ml the maximum reducing power was observed (62%). A strong scavenging ability of DPPH (79.4%) was observed for IONPs at a concentration of 200 µg/ml. Based on the results shown in Figure 9, it can be inferred that a number of antioxidant chemicals may have a role in the IONPs' stabilisation and decrease when *Rhamnus virgata* leaf extract is used. Our findings are consistent with earlier research on biogenic IONPs that used Fagonia indica and Callistemon viminalis [16, 13].

Protein kinase inhibition

As mentioned above, a protein kinase (PK) inhibition experiment for green IONPs was performed using the Streptomyces 85E strain [2]. The entire experiment was performed under aseptic conditions. Streptomyces strain 85E grassland could also be produced using the SP4 minimum soil. Then 10 µl of IONP was applied to the microbial mats in combination with autoclaved 6 mm filter

discs. We used surfactin and DMSO as positive and negative controls, respectively. In order to control the growth of the strain, an incubation period of 72 hours at 30 °C was carried out. Spore and mycelium production was inhibited as evidenced by the presence of clean, bare areas around the intervertebral discs. Zones of inhibition in millimeters were measured with a vernier caliper [11].

Alpha amylase inhibition

Using a previously refined technique, the alpha-amylase (AA) inhibitory activity of biogenic IONPs was determined *in vitro* [17]. Stepwise dilutions of 25 µl AA enzyme, 40 µl starch solution, 10 µl test sample and 15 µl phosphate buffered saline were combined to form a reaction mixture. 20 µl (1 MHCCl) and 90 µl of iodine solution were added to the reaction mixture with all components, and this was then placed in an incubator at 50 °C. For 30 minutes. Acarbose and distilled water were used as positive and negative controls, respectively [11].

Enzyme inhibition potential

The effectiveness of biogenic IONPs in preventing PK enzymes is shown in Figure 10. PKs are enzymes that control multiple physiological processes including the metabolism, differentiation, proliferation, and apoptosis of phosphorylated tyrosine and serine residues/threonine. Dysregulated phosphorylation can lead to genetic abnormalities that cause cancer. Therefore, any substance that can inhibit PK enzymes could be of crucial importance in the treatment of cancer [31]. Since PK phosphorylation has a significant impact on hyphae formation in the Streptomyces fungal strain, the same principle is used to investigate the possibility of PK inhibition. Mainly the strain was used to find pharmaceuticals to test PK inhibition [32].

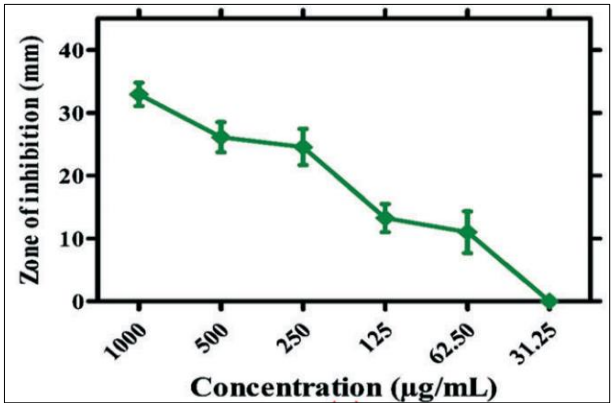

Fig 10: Iron oxide nanoparticles (IONPs) biocompatibility, antioxidant, and enzyme inhibition assays. (IONPs). The potential of inhibiting protein kinase (PK).

Surfactin was used as a positive control in the PK inhibition test using the disk diffusion technique in the concentration range from 1000 to 31.25 µg/ml. At 1000 µg/ml, inhibition bands of up to 32 mm per IONP were detected, indicating that biogenic IONPs have a high ability to inhibit PK. We decide that the answer depends on the dose. This will produce a potent signal transduction inhibitor in nanoscale IONPs, which will be further tested for its anticancer and anti-infective properties. Presumably, biogenic IONPs would play an important role in cancer research due to their propensity for PK inhibition [11].

Additionally, *Rhamnus virgata*-inspired IONPs were tested for AA inhibition activity at concentrations ranging from 1000 to 31.25 g/ml.The primary focus of diabetes research is on AA, which changes carbohydrates into glucose [31] and whose action may be blocked in order to decrease blood glucose levels [33, 34]. It was determined how well-bioinspired IONPs inhibited AA. Our findings showed that IONPs were efficient at inhibiting AA. The highest recorded concentration of 1000 µg/ml is stated to have a maximum inhibition of 21%. However, when sample concentration was lowered, the maximum inhibition steadily declined. The ability of green IONPs to block AA is seen in Fig 11 [11].

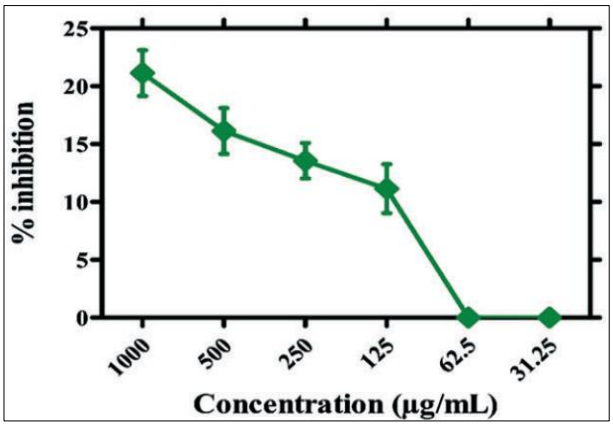

Fig 10: Iron oxide nanoparticle (IONP) biocompatibility, enzyme inhibition, and antioxidant tests. Restraint potential against alpha amylase (AA).

Conclusion

Although the plant is well known for its medicinal properties, controlled clinical trials are necessary to prove and determine its real efficacy. Additional research for the isolation and characterization of molecules could lead to drug discovery via natural sources. The mysterious and miraculous tree, *Rhamnus virgata*, has been demonstrated for its broad spectrum of applications in the management of several ailments.

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