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RSM optimized *Moringa oleifera* peel extract for green synthesis of *M. oleifera* capped palladium nanoparticles with antibacterial and hemolytic property

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ABSTRACT

Palladium nanoparticles (Pd NPs) are the very good catalytic agents in many coupling reactions, also these are very well biological agents against bacteria and fungus. *M. oleifera* capped Pd NPs were synthesized from microwave assisted methanolic extract of *M. oleifera* peel. To optimize the extraction process RSM (Response Surface Methodology) was applied. To get a good extraction yield BBD (Box-Behnken Design) was employed. The better optimized conditions for the extraction was found as 400 W, 25 mL of CH_3OH at 65 °C for 2 min. We observed 61.66 mg of extract yield from this method. Eco-friendly *M. oleifera* capped Pd NPs were synthesized using *M. oleifera* peel extract and confirmed using the different characterization techniques like UV- Vis spectroscopy, XRD, SEM and HR-TEM analysis. We found the size of the *M. oleifera* capped Pd NPs nanoparticles as 27 ± 2 nm and shape of the particles as spherical through the TEM analysis. *M. oleifera* capped Pd NPs exhibits good antibacterial activity against *S. aureus* (*Staphylococcus aureus*) and *E. coli* (*Escherichia coli*) bacterial strains and we found the zone inhibition as 0.6 and 0.7 mm. The synthesized *M. oleifera* capped Pd NPs are screened for hemolytic activity and it proved the *M. oleifera* capped Pd NPs are non-toxic on RBCs cells.

1. Introduction

Plant species have been considered as one of the best reservoirs of many pharmaceuticals. Recently, the plant derivatives were well derived and used in medicinal field [1]. *M. oleifera* is a one of the important and easily available medicinal plant which cultivated in tropic and sub tropic area of Asia and Africa countries [2]. Different parts of the plant (leafs, flowers and roots) are traditionally used for the dietary purpose [3]. The leaf powder was used as the best treatment for malnutrition in children, pregnant women and nursing mothers. Also, it is rich in vitamins content, minerals like calcium, iron, potassium and protein [4]. The seeds of the *M. oleifera* were reported to be acrid, bitter and also these are good antipyretic, antimicrobial agents [5]. This plant controls the many diseases in humans due to the unique properties of this plant play a major role in controlling the health diseases in human being. Polypeptides which are present in it act as the natural coagulants for treatment of water [6].

In the last few decades the extraction process has been extensively investigated and focused mainly on the conventional method. Different extraction methods have been reported, such as Soxhlet extraction, pressurized liquid extraction, ultrasound mediated extraction technique, reflux extraction and supercritical fluid extraction [7]. MW (microwave) methodology has been considered as one of the excellent method to extract the bioorganic phase also it can reduce the time and solvent consumption [8].

RSM has been considered as one of the developed method by Box and Wilson in the year of 1951. Extraction process depends on the plant material, irradiation time, solvent, pressure, temperature, and other factors. The effectiveness of these factors on extraction process can optimize individually by RSM technique [9]. There are different types of RSM techniques were present to optimize the variables such as three level factorial technique, central composite technique and Box-Behnken Design [10].

Nanoparticles synthesis has been depend upon the different methods like physical methods, and chemical methods. Among these green synthesis is a favorable method for the synthesis of metal and metal oxide nanoparticles [11]. Plant source mediated nanoparticles synthesis is one of the attracting tool from last few decades due to the less cost and nontoxic [12]. Plant extract can play main role as a capping or reducing agent in nanoparticles synthesis [13]. The nanotechnology is focusing the synthesis high potency transition metal nanoparticles due to their effectiveness in catalytic approach [14,15]. Pd NPs are

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having higher catalytic surface and these nanoparticles are good in biological values. There are various documents are obtainable for Pd NPs production with help of plant extracts [16–21]. In the present work, first time we have used the RSM optimized *M. oleifera* peel methanol extract for the production of *M. oleifera* capped Pd NPs. We have determined the antibacterial and hemolytic activity of the synthesized *M. oleifera* capped Pd NPs.

2. Materials and Methods

2.1. Collection of Drumstick [M. oleifera] Peel

The peel of drumstick was collected and processed as per our earlier method [22].

2.2. Microwave Assisted Extraction Process

Above 1 g of *M. oleifera* peel powder sample in methanol was placed in microwave (Uwave 1000, 220 V/50 Hz, Sineo microwave, UV, US synthesis extraction reactor, China). Different variables such as microwave power (300, 400 and 500 W), Temperature (60, 65 and 70 °C) extraction time (1, 2 and 3 min), solvent quantity (20, 25 and 30 mL/g) of methanol portion (100%) were applied for obtaining good yield of extract. The extraction process was explained detailed in systematic sketch (Fig. S1).

2.3. The RSM Design for the Extraction of M. oleifera Extract

To find out the appropriate extraction condition for the *M. oleifera* peel extract, RSM methodology was used. Symmetrically, there are four levels which were used with the 29-run BBD quadratic model. This was utilized to optimize the effectiveness of the each variable such as microwave power (W, A), temperature (°C, B), irradiation time (min, C) and solvent to solid ratio (mL, D).

The quadratic polynomial equation [23] was used to the find suitable variable described as below.

$$Y = \beta 0 + \beta 1\,(A) + \beta 2\,(B) + \beta 3\,(C) + \beta 4\,(D) + \beta 11\,(A2) + \beta 22\,(B2) + \beta 33\,(C2) + \beta 44\,(D2) + \beta 12\,(AB) + \beta 13\,(AC) + \beta 14\,(AD) + \beta 23\,(BC) + \beta 24\,(BD) + \beta 34\,(CD)$$

where, β0 is the constant co-efficient of the model, (β1, β2, β3, β4), (β11, β22, β33, β44) and (β12, β13, β14, β23, β24 and β34) is regression coefficients, A (watts), B (temp), C (min) and D (solvent ratio) are coded variables.

2.4. Synthesis of Pd NPs Using M. oleifera Peel Extract

Microwave irradiation method was followed for the synthesis of Pd NPs, in this process $Pd(OAc)_2$ (1 mM, 80 mL) solution was mixed with the 20 mL of RSM optimized methanolic extract of *M. oleifera* peel. The reaction mixture was placed in the microwave cabin and allowed to irradiation process at 300 W for 5 min. The centrifugation was carried out to remove the nanoparticles from reaction mixture using 4000 rpm (15 min). The calcination procedure was employed 300 °C. The *M. oleifera* capped Pd NPs was stored in refrigerator for the additional use.

2.5. Characterization of M. oleifera Capped Pd NPs

The *M. oleifera* capped Pd NPs formation was confirmed using absorbance of UV–Vis spectroscopy (HitachiU2910 spectrophotometer). Functional groups which are present in the extract and *M. oleifera* capped Pd NPs were analyzed using FT-IR spectroscopy (SHIMAZDU infrared spectrophotometer). The XRD analysis (Bruker-Germany, model D8) was applied to find the crystalline nature of the *M. oleifera* capped Pd NPs. The shape was determined using SEM analysis (JEOL Model JSM - 6390LV) and size of the particles was determined using TEM analysis (Philips, CM 200).

2.6. Antibacterial Efficiency of M. oleifera Capped Pd NPs

The *S. aureus* and *E. coli* bacterial cultures were used for the identification of the antibacterial capability of *M. oleifera* capped Pd NPs. The method which we adopted for this study was well diffusion method; bacterial strains were spread on the nutrient agar media. The synthesized *M. oleifera* capped Pd NPs (25 μL) were taken and inoculated in the 7 mm diameter of the well which was made by well borer. After the inoculation, the Petri dishes were incubated at room temperature for 24 h [24]. Amoxicillin–1 was used as positive control for the activity.

2.7. Hemolytic Assay of M. oleifera Capped Pd NPs

The cell lysis capacity or toxicity on RBCs of the synthesized *M. oleifera* capped Pd NPs was determined according the existed protocol [22]. The B + Ve blood has been collected in a sterile container from the 25 age old specimen. Blood sample was allowed to centrifuge (10 min, 1500 rpm) to get the supernatant and the process was repeated thrice by adding PBS (phosphate-buffered saline) at pH 7.4 for obtaining pure pellet. Four different concentration of *M. oleifera* peel extract such as 25, 50, 75 and 100 μL were added to the blood sample and final volume was made up to 1 mL using PBS. The sample was kept 60 min at RT (room temperature) for incubation. Further, the sample was centrifuged (10 min, 1500 rpm) and the absorbance of the collected supernatant was measured (540 nm). Triton–X 100 and blood sample with PBS have been used as positive control and negative controls. The percentage hemolysis was calculated as following formula,

$$(A_s - A_{nc} / A_{pc} - A_{nc}) * 100$$

where,

A_s	absorbance of sample
A_{nc}	absorbance of negative control
A_{pc}	absorbance of positive control.

3. Results and Discussion

3.1. Response Surface Analysis of Extraction Process

The multi non-linear model plots which are having three dimensional interactions were plotted to determine the interactive effects and reciprocal interactions of the independent variables on the extraction process shown in Figs. 1 and 2. These non-linear plots were made at the Z-axis to find out the two independent variables such irradiation power and solvent ratio while measuring other two independent variables such as irradiation time and temperature at the zero level using their response [25].

3.1.1. Influence of Irradiation Power on Extraction Yield

The extraction yield of *M. oleifera* peel was investigated by the effect of irradiation power. The microwave power ranging from 300 to 500 W with different quantity of methanol solvent and irradiation time (1–3 min) have been used for this study. A significant increase of extraction yield 61.66 mg was observed at the power of 400 W. Similarly, the microwave power was influenced the effectiveness of the extraction yield, as a results <400 W suggested to the fine irradiation power for the extraction process [26], Based on these observations 400 W has been considered as suitable condition to extract process.

3.1.2. Influence of Irradiation Time on Extraction Yield

In this study, the effect irradiation time (1–3 min) has been carried out for the determination of extraction yield using other