An Eco-friendly Green Synthesis of Tungsten Nanoparticles from Moringa oleifera Lam. and Their Pharmacological Studies

Moringa oleifera Lam’dan elde edilen Tungsten Nanopartiküllerinin Çevre Dostu Yeşil Sentezi ve Farmakolojik Çalışmalar

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ABSTRACT

Integration of nanoscience in medicine leads to the development of biomedicinal products that helps the society in a faster and safer manner. In the present studies tungsten nanoparticles were synthesized by green route using aqueous extracts of Moringa oleifera. Characterization was done through UV, SEM, TEM, FT-IR and XRD. The synthesized nanoparticles were spherical in shape with an average size of 10 nm. Various bioactive compounds present in aqueous extract of this plant were responsible for bio reduction of nanoparticles. Further these nanoparticles were tested for various biological activities. In antimicrobial activity, it was observed that potent activity was shown against Bacillus subtilis (18mm) at 80µg/ml while against fungus maximum activity was observed against Fusarium oxysporium (20mm) at same dose. The platelet aggregation of nanoparticles was assayed by Prothrombin (PT) and Activated Partial Thromboplastin time (APTT). In PT assay maximum activity was observed at 100 µg/ml (158 sec) and in APTT it was found at 60 µg/ml (120sec). Cytotoxicity was also studied by MTT assay against various cell lines. Against MCF-7 (Breast cancer cell line) nanoparticles were active at 200 µg/ml while in 3T3 (fibroblast cell line) they were potent at 500 µg/ml. Result showed the biosynthesis of tungsten nanoparticles using aqueous extract of Moringa oleifera is a clean, inexpensive and safe method that is free from toxic substance and consequently does not have any side effects.

Key Words: Green synthesis; Moringa oleifera; Platelet aggregation; Cytotoxic Assay; Antimicrobial activity.

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ÖZET

Nanobilimin tıbbi entegrasyonu, topluma daha hızlı ve daha güvenli bir şekilde yardımcı olan biyomedikal ürünler geliştirilmesine yol açar. Mevcut çalışmalarında tungsten nanopartiküller, Moringa oleifera'nın sulu ekstraktı kullanılarak yeşil yolla sentez edilmiştir. UV, SEM, TEM, FT-IR ve XRD ile karakterizasyon yapıldı. Sentezlenen nanopartiküller, ortalamı 10 nm'lik bir boyutu ve küresel şekile sahipti. Bu bitkinin sulu ekstraktı bulunan çeşitli biyaktivite bileşikleri, nanopartiküllerin biyolojik olarak indirgememeden sonuçlandı. Ayrıca bu nanopartiküller, çeşitli biyaktivite aktiviteler için test edildi. Antimikrobiyal aktivitete, aynı dozda Fusarium oxysporium'a (20 mm) karşı funguslara karşı maksimum aktivite gösterdi. Sonuç, Moringa oleifera'nın sulu ekstraktı kullanılan中新网 nanoparticles, sahip olduğu toksik malzeme temizdir. MCF-7'ye (Breast cancer hücreli) karşı MTT testi ile incelenmişti. Moringa oleifera'nın sulu ekstraktı kullanılarak sentezlenen nanopartiküllerin biyosentezinde toksik madde içermeyen temiz, ucuza ve güvenli bir yöntem olduğunu ve dolayısıyla herhangi bir yan etkisi olmadığı gösterdi.

Anahtar Sözcükler: Yeşil sentez; Moringa oleifera; Trombosit agregasyonu; Sitotoksik Deney; Antimikrobiyel etkinlik.

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INTRODUCTION

Nanotechnology is described as portents at nanometre scale which is generally stated in range of 1-100 nm. It manipulates various substances at the atomic, molecular along with macromolecular level to innovate substances at nanometre scale, and systems that have unique features and functions. The eco-friendly approach by green route of synthesis of nanoparticles is easy, efficient, in comparison to chemically synthesised or through use of microbes. Plant extracts could be an alternative to conventional chemical routes for the synthesis of metallic nanomaterials in a clean, nontoxic and ecologically sound manner (1). The key advantages green synthesis route is that they possess significant phytochemicals which helps in the reduction of tungsten ions. These phytochemicals include ketones, aldehydes, amides, terpenes, flavones, which are directly related to the reduction of nanotungsten oxide particles.

Moringa oleifera Lam is categorized in Moringaceae family having 14 species among which M. oleifera is most commonly found. In India conventionally this plant is known by the name “Sahanyana”. Its pods and leaves have rich source of biopolymers like it contain 2.5 and 6.7 g protein/100 g, respectively. The plant is also recommended in folk remedies for conjunctivitis, high blood pressure, abdominal, boils, cold, discomfort, tumors, relapsing fever, hysteria and skin diseases, etc. It also bears certain bioactivity viz., anti-inflammatory, anti-arthritic, antioxidant and hepatoprotective, antitumor etc. Moringa oleifera is consumed in Asian diet since many decades as raw food source.

The characterization and coating of tungsten oxide nanofibers by electrospinning process and sol-gel technique has been recommended by many researchers (2). They initiated the potential importance of the nanofibers as a sensor material for gas detection. Ultrafine tungsten and tungsten oxide powders with determined particle size and structure had been processed by a reverse microemulsion-mediated protocol (3). The fascinating solicitations in different fields like electronics, illumination, catalysis and gas sensors has been reported.

Recently existence and banquet of antimicrobial resistance is a serious issue in both developing and developed nations and which can leads to global crisis (4). A stratagem for the repression of resistance needs to be innovated, executed and evaluated which should be focused on improving rational use of antimicrobials and reducing prospects for spread of resistant organisms (5) It has been reported (6) that, the metallic nanoparticles are meticulously being explored and broadly explored as potential antimicrobials. The antimicrobial potency of the nanoparticles is known to be a function of the surface area in communication with the microorganisms. Therefore, the search for new antimicrobial drugs from nanoparticles derived from natural sources has increased as a substitute to commercial drugs.

Arterial thrombosis swayed by aggregation of platelet are responsible for life-threatening disorders like unstable angina and reocclusion after angioplasty. So adjournment of platelet aggregation is essential physiologically for prevention and treatment of cardiovascular diseases (7). During the initial stage of thrombosis, damage in blood vessels causes the production of adhesive proteins (such as collagen and von Willebrand factor) and soluble agonists (such as ADP and thrombin) at the injury site; which further leads to platelet adhesion, activation, and aggregation, therefore leads to formation of a platelet-rich thrombus (8).

It has been reported that synthesis of nanoparticles from biological sources is of keen interest due to synergistic properties valued by such nanoparticles. Heparin (HP), when composited with nanomaterials, has been of keen interest for its chemical and biological features. HP has a number of therapeutic applications which can be elevated when composites with nanoparticles and has been recommended in various biological applications (9).

Biologically cancer is an undifferentiated growth of mass of cells bearing uncontrolled cell division (10) which is a major serious issue globally (11). So there is urge need for anticancer therapy (12). The fight against cancer is difficult particularly in the development of therapies for severely multiplying tumors. Chemotherapy is available for treatment of cancer but still it has low specificity and is limited by dose. It is a challenge to find the therapy and drugs for the treatments of various types of cancer. So, conventional process urge for the amalgamation of controlled released process and targeted drug delivery which is more effective and less harmful. Nanomaterials are being recommended at global level for cancer diagnosis and therapy (13).

Considering the importance of nanoparticle metal synthesis especially tungsten using different plants, the aim of this study was green synthesis of tungsten nanoparticles using aqueous extract of M. oleifera and investigation of its antimicrobial, antioxidant, antiplatelet, cytotoxicity and anti-tuberculosis activity.

MATERIALS and METHODS

The chemicals used for the synthesis of tungsten nanoparticles are tungsten oxide WO3 which was purchased from Hi-media, Jaipur, India. Doubly distilled water was used for the preparation of aqueous extracts. In vitro assay reagents for antimicrobial, antiplatelet and anticancer activity were purchased from Sigma Aldrich, India.

Synthesis of Tungsten nanoparticles

The synthesis of nanoparticles via green route processes were synthesised biologically by aqueous extract of Moringa oleifera. Initially leaves were washed properly with distilled water (14). 1mM of tungsten oxide was taken in 100ml autoclaved distilled water in a conical flask and kept on magnetic stirrer. 50 gm leaves were weighed and grinded properly in the mortar and pestle for 15 to 20 minutes in distilled water. These grinded leaves were centrifuged at 4°C, 10000rpm for 5minutes. After the centrifugation the supernatant was collected and added to the flask containing the solution of tungsten oxide. It was kept overnight and the change in the colour of the solution from light brown to dark brown was observed which indicates the reduction and synthesis of tungsten nanoparticles.

Characterisation

Synthesised nanoparticles were characterised using various techniques viz, UV, SEM, TEM, FT-IR and XRD and further tested for their biological assays.

Antimicrobial activity

Antimicrobial activity of the synthesised nanoparticles was investigated by agar well diffusion method (15, 16,17) and activity index was calculated as:

\[
\text{ACTIVITY INDEX} = \frac{\text{Zone of inhibition of sample}}{\text{Zone of inhibition of standard}}
\]

Antiplatelet Activity

Blood samples were collected from KCI Diagnostic center, near SMS Medical College, Jaipur and subjected to centrifugation. Centrifugation at 10000 rpm for 5.5 min, 0.2 ml platelet rich plasma was separated from the sample, dissolved in isotonic CaCl2. Different hemostatic constrains viz. Prothrombin time (PT) and Activated partial thromboplastin time (APTT) were investigated using protocol of Perkin (18).

Cytotoxic assay

MCF-7 cells are widely used cell lines in human breast cancer. They are derived from a pleural effusion of a malignant breast cancer and having estrogen and progesterone receptors. The human breast cancer cell line, Michigan Cancer Foundation-7 (MCF-7) and fibroblast cell line 3T3 was received from National Centre for Cell Science at Pune. The cell lines fed in 10% fetal bovine serum (FBS) accomplished with Eagle minimum essential medium. Further they were maintained with laboratory conditions with 95% air and 100% relative humidity at 37°C in 5% CO2. The viable cells noted down using a hemocytometer and diluted with medium having 5% FBS to give cell count of 1 X 10^5 cells/mL. The cytotoxicity evaluation of Moringa oleifera extract stabilized tungsten nanoparticles was performed using tetrazolium dye MTT assay as described by (19). About 1 x 10^3 mL^1 cells of MCF-7 and 3T3 cell lines in their experimental growth phase was seeded in a flat bottomed 96 well polystyrene coated plate and incubated for cell attachment.

Statistical analysis

All the quantitative experiments were carried out in replicates, mean and standard deviation were calculated and results are expressed as means ± standard deviation.
RESULTS

Green synthesis of Tungsten nanoparticles

The *Moringa oleifera* plant extract was employed for the green synthesis of tungsten nanoparticles using established protocols.

After the addition of the plant leaf extract to solution of tungsten oxide it was observed that the colour of the reaction mixture was gradually changed from light yellow to dark brown, indicating the synthesis of nanoparticles (Fig. 1).

Characterization of synthesised nanoparticles

**Ultraviolet-visible spectroscopy**

Formation of nanoparticles is generally determined by optical properties which is one of the main criteria for their synthesis. Free electrons in the these nanoparticles educed by gripping visible light and transmitted to a higher energy level but the electron is unstable in an excited state and returns to the base energy level and as a result a photon is emitted. Simultaneously resonance frequency of surface plasmon in the metallic nanoparticles depends on shape, size and environment maintained during synthesis of nanoparticles. The UV-Vis spectrum of tungsten nanoparticles gave absorbance peaks around 400 nm and it showed strong resonance at this wavelength. The UV-vis spectra also revealed that these nanoparticles remained stable even after 24 h and it further confirms the biosynthesis of nanoparticles. (Fig. 2)

**SEM and TEM**

SEM was applied to study surface morphology and the topography of synthesized nanoparticles. The size of nanoparticles varied from 10 to 20 nm. It was observed that the biosynthesized nanoparticles were mostly spherical in shape. The shape and size of the nanoparticles were further studied by TEM. It was observed that synthesized nanoparticles were well dispersed, with an average size of 10 nm. The data from TEM image were in a good agreement with the SEM. (Fig. 3 and 4).
FT-IR

FT-IR was applied in order to investigate possible biomolecules bearing different functional groups responsible for reduction, capping and efficient stabilization of newly synthesized nanoparticles (Fig. 5). The absorption bands at 3340, 2948, 2821, 2042, 1662, 1459, 1402, 1314, 1108 and 949 cm⁻¹ were observed. The strong peaks at 3340 cm⁻¹ corresponds to Hydroxy group, H-bonded OH stretches. The band at 2948 cm⁻¹ was attributed to methyl CH asym./sym stretches. The peak at 2821 cm⁻¹ corresponds to methyl amino, N-CH₃ and C-H stretches.

The band at 2042 cm⁻¹ corresponds to transition metal carbonyl, while band at 1662 cm⁻¹ corresponds to alkenyl C=CH stretch, band at 1459 shows presence of methylene C-H bends. Band at 1402 cm⁻¹ corresponds to phenol or tertiary alcohol, OH which is also represented by bands at 1314 cm⁻¹ while 1108 cm⁻¹ represents secondary alcohol having C-O stretches and 949 cm⁻¹ represents C-H out of plane bend. These different phytochemicals were responsible for biosynthesis of tungsten nanoparticles.

\[ D = \frac{K \lambda}{\theta \cos \theta} \]

where \( D \) is the average crystalline diameter size (Å), \( K \) is a constant (0.9), \( \lambda \) is the wavelength of the X-ray used (\( k = 1.54 \text{ Å} \)), \( \theta \) is the angular line width at the half maximum of diffraction (radians) and \( '\theta' \) is the Bragg’s angle (degrees) (20).

**Figure 5** FT-IR spectra showing presence of different functional groups engaged in synthesis of nanoparticles

**XRD**

X-ray diffraction (XRD) studies were carried out to confirm the synthesis of WNPs and characterize crystallinity and the phase pattern of synthesized tungsten nanoparticles.

It was observed that 2θ (in degrees) were in the range of 28.4 to 66.5°C (Fig. 6). The said 2θ values of peaks were in accordance with the standard of JCPDS. The XRD study confirms that the resultant particles were nanoparticles. Furthermore, it also confirms that the synthesized nanoparticles were free of impurities as no other characteristics XRD peaks were observed. The mean grain crystalline size of green synthesized tungsten nanoparticles was calculated using the Debye–Scherrer formula.

![Figure 6 XRD spectra showing diffraction pattern of synthesized nanoparticles.](image)

**Table 1** Antibacterial activity of Tungsten Nanoparticle prepared by green synthesis route

| Concentration (in µg/ml) | E.coli | Bacillus subtilis | Pseudomonas aeruginosa | Streptomyces griseus |
|--------------------------|--------|------------------|------------------------|---------------------|
| 20                       | IZ-Nil | IZ-Nil           | IZ-12 ±0.03            | IZ-8 ±0.009         |
| 40                       | Al-    | IZ-6±0.008       | Al-0.55                | Al-0.33             |
| 60                       | Al-0.27| IZ-13±0.03       | IZ-Nil                 | IZ-Nil              |
|                         | Al-0.59| IZ-Nil           |                        | IZ-Nil              |
| 80                       | Al-0.55| IZ-Nil           | IZ-12±0.03             | IZ-10±0.01          |
| Standard                 | IZ-22  | IZ-18±0.06       | IZ-14±0.04             | IZ-16±0.06          |

* Ciprofloxacin (as Standard at 1mg/ml), IZ- Inhibition Zone (in mm), Al- Activity index (Values are mean of three replicates)

**Antimicrobial activity**

The reduced nanoparticles showed potent antibacterial and antifungal activity at concentration ranging from 20µg/ml to 80µg/ml on various clinical isolates. It was observed that against *E. coli* maximum zone was observed at 80µg/ml (14mm). Against *Bacillus subtilis* maximum zone was observed at 80µg/ml (18mm) which was highest among all tested sample. When nanoparticles were tested against *Pseudomonas aeruginosa* it was observed that no activity was observed at 40 and 60 µg/ml while maximum activity was observed at 80 µg/ml (14mm) which was at par with *E. coli*. Against *Streptomyces griseus* maximum zone was observed at 80 µg/ml (16mm) (Table 1).

In antifungal activity maximum activity was observed at 80 µg/ml (20mm) against *Fusarium oxysporum*. Against *Penicillium funiculosum* activity was observed at only 60 and 80 µg/ml (14 and 16 mm respectively). Against *Candida albicans* maximum activity was observed at 80 µg/ml (16mm) which was at par with that of *Penicillium funiculosum*. However *Trichoderma reesei* was found to be partial resistant (Table 2).
The present study revealed the anticancer and cytotoxic potential of nanoparticles on breast cancer cells MCF-7 and fibroblast cell line 3T3 and the report was compared with cisplatin at various concentrations. It was noticed that nanoparticles with a concentration ranging from 10 to 200 µg/ml resulted in dose dependent decrease in cellular viability of cancer cells with IC50 value of 44.79 µg/ml while cisplatin treatment revealed IC50 value of 9.47 µg/ml.

The variation between the positive drug and samples is because the positive drug is a pure and so it will require lower concentration to inhibit the growth of cancer cells. Alternatively, higher concentration of samples resulted in more than 50% inhibition of cancer cells. Screening of cytotoxicity of nanoparticles on 3T3 cells revealed that it was marginally toxic to cells even at higher concentration.

**Antifungal Activity**

| Concentration (in µg/ml) | Fusarium oxysporum | Penicillium funiculosum | Candida albicans | Trichoderma reesei |
|--------------------------|---------------------|------------------------|------------------|-------------------|
| 20                       | IZ-6±0.02           | IZ-Nil                 | IZ-Nil           | IZ-Nil            |
| 40                       | IZ-14±0.04          | IZ-Nil                 | IZ-10±0.01       | IZ-8±0.008        |
| 60                       | IZ-18±0.08          | IZ-14±0.04             | IZ-14±0.04       | IZ-10±0.01        |
| 80                       | IZ-20±0.1           | IZ-16±0.06             | IZ-16±0.06       | IZ-12±0.02        |
| Standard                 | IZ-20               | IZ-20                  | IZ-20            |                   |

* Ketokazole (as Standard at 1mg/ml), IZ- Inhibition Zone, AI- Activity index (Values are mean of three replicates)

**Activated Partial thromboplastin time (APTT)**

In this assay significant activity was observed at 60µg/mL (2.55 times of control and 3 times as compared to standard), which increased slowly (Table 4) with increase in dose level.

**Prothrombin Time (PT)**

All the concentrations of nanoparticles prolonged the clotting time as compared to control. Significant activity was observed at 100µg/mL (4.27 times of control and 10.5 times as compared to standard), which was maximum and increased in linear fashion according to dose level (Table 3).

**Table 2 Antifungal activity of Tungsten Nanoparticle prepared by green synthesis route**

| S.N | Concentration of Sample (in µg/ml) | Time | Inhibition Zone, AI | Activity index, IZ |
|-----|-----------------------------------|------|---------------------|-------------------|
| 1   | 10                                | 25 sec | 1.66               | 0.67              |
| 2   | 20                                | 58 sec | 3.86               | 1.56              |
| 3   | 30                                | 40 sec | 2.66               | 1.08              |
| 4   | 40                                | 45 sec | 3.0               | 1.21              |
| 5   | 50                                | 88 sec | 5.86              | 2.37              |
| 6   | 60                                | 80 sec | 5.33              | 2.16              |
| 7   | 70                                | 85 sec | 5.66              | 2.29              |
| 8   | 80                                | 102 sec | 6.8             | 2.75              |
| 9   | 90                                | 135 sec | 9.0            | 3.64              |
| 10  | 100                               | 158 sec | 10.5            | 4.27              |

Control – 37 s.
Standard PT (plasma + PT reagent) – 15 s.
* Denotes potency of the sample at different concentrations when compared with standard and control

**Table 3 Antipatelet activity of reduced Tungsten nanoparticles by Prothrombin time (PT)**

| S.N | Concentration of Sample (in µg/ml) | Time | * Standard | * Control |
|-----|-----------------------------------|------|------------|-----------|
| 1   | 10                                | 45 sec | 1.21       | 0.95      |
| 2   | 20                                | 37 sec | 0.92       | 0.78      |
| 3   | 30                                | 55 sec | 1.37       | 1.17      |
| 4   | 40                                | 62 sec | 1.55       | 1.31      |
| 5   | 50                                | 75 sec | 1.87       | 1.59      |
| 6   | 60                                | 120 sec | 3.0        | 2.55      |
| 7   | 70                                | 58 sec | 1.45       | 1.48      |
| 8   | 80                                | 115 sec | 2.55      | 1.70      |
| 9   | 90                                | 60 sec | 1.33       | 1.27      |
| 10  | 100                               | 54 sec | 1.35       | 1.14      |

Control – 47 s.
Standard PT (plasma + PT reagent) – 40 s.
* Denotes Potency of the sample at different concentrations when compared with standard and control

**Table 4 Antipatelet activity of reduced Tungsten nanoparticles by Activated Partial Thromboplastin time (APTT)**

| S.N | Concentration of Sample (in µg/ml) | Time | * Standard | * Control |
|-----|-----------------------------------|------|------------|-----------|
| 1   | 10                                | 45 sec | 1.21       | 0.95      |
| 2   | 20                                | 37 sec | 0.92       | 0.78      |
| 3   | 30                                | 55 sec | 1.37       | 1.17      |
| 4   | 40                                | 62 sec | 1.55       | 1.31      |
| 5   | 50                                | 75 sec | 1.87       | 1.59      |
| 6   | 60                                | 120 sec | 3.0        | 2.55      |
| 7   | 70                                | 58 sec | 1.45       | 1.48      |
| 8   | 80                                | 115 sec | 2.55      | 1.70      |
| 9   | 90                                | 60 sec | 1.33       | 1.27      |
| 10  | 100                               | 54 sec | 1.35       | 1.14      |

Control – 47 s.
Standard PT (plasma + PT reagent) – 40 s.
* Denotes Potency of the sample at different concentrations when compared with standard and control

**Cytoprotective Assay**

The present study revealed the anticancer and cytotoxic potential of nanoparticles on breast cancer cells MCF-7 and fibroblast cell line 3T3 and the report was compared with cisplatin at various concentrations. It was noticed that nanoparticles with a concentration ranging from 10 to 200 µg/ml resulted in dose dependent decrease in cellular viability of cancer cells with IC50 value of 44.79 µg/ml while cisplatin treatment revealed IC50 value of 9.47 µg/ml.
Overall in MCF-7, the viable cells were around 65% at 100µg/ml which decreased to 47.20% which reveals the fact that it is toxic at increasing concentration of test sample. In 3T3 cell lines, it was observed that the cells were viable at 61% at 500µg/ml which proved its non-toxicity (Tables 6-8).

### Table 6 Showing percent cell viability of standard (Cisplatin) (MCF7 breast cancer cell line)

| Concentrations(µg/ml) | Viability |
|-----------------------|-----------|
| 5                     | 57.02±0.43 |
| 10                    | 52.12±0.34 |
| 25                    | 44.52±0.26 |
| 50                    | 41.09±0.33 |
| 100                   | 23.52±0.12 |
| 250                   | 19.36±0.7 |
| 500                   | 9.47±0.5 |

### Table 7 Showing cell viability of tungsten nanoparticle against MCF7 breast cancer cell line.

| Tested concentrations | Viability |
|-----------------------|-----------|
| 10                    | 92.49±0.65 |
| 25                    | 85.21±0.58 |
| 50                    | 78.59±0.44 |
| 100                   | 65.42±0.37 |
| 200                   | 44.79±0.43 |
| Control               | 100       |

### Table 8 Percent cell viability of tungsten nanoparticle against 3T3 fibroblast cell line

| Tested concentrations(µg/ml) | Viability |
|-----------------------------|-----------|
| 25µg                        | 94.39±0.88 |
| 50 µg                       | 88.12±0.76 |
| 100 µg                      | 75.30±0.73 |
| 250 µg                      | 67.76±0.70 |
| 500 µg                      | 61.29±0.65 |
| control                     | 100.00    |

**DISCUSSION**

Nanotechnology is increasing at rapid rate in biomedical sciences as innovative techniques which are being developed to investigate and manipulate the effect of single atoms and molecules against a wide range of living cells. The current investigation presents a systematic and scientific approach to develop and investigate the nanoparticles and its biological activities against a range of routes. There is a great scope of the study of nanoparticles and as well as natural products in the present time a range of routes. There is a great scope of the study of nanoparticles and as well as natural products in the present time a range of routes. There is a great scope of the study of nanoparticles and as well as natural products in the present time.

There are some earlier reports which proved that (21) anti-bacterial activity of the silver nanoparticles synthesized via green route has potency against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella* spp. Further some researchers (22) reported that the silver nanoparticles had effect on antimicrobial and antifungal activity of new heterocycles. Synergetic effects of the silver nanoparticles on the antimicrobial activity were reported by disk diffusion method. These silver nanoparticles possess potent activity against *Aspergillus flavus* which were observed in case of tungsten nanoparticles against selected microbes (23).

The strong antimicrobial effects in natural products synthesize by plants are very crucial as the resistance of many pathogens to antibiotics is one of the serious issues of medical science. Dependence on antibiotics result in raising the cost of health care due to prolonged treatment like admission and restoration, need to the making of new antibiotic agents and applying effective and widespread methods of infection control in order to prevent the spread of pathogens resistant to antibiotics. Some issues when consuming antibiotics is the occurrence of lethal and dangerous side effects such as hypersensitivity reactions, growth inhibition of hematopoietic stem cell, liver and kidney failure in some of patients. With the innovations of nanotechnology and production of plant based nanoparticles for their therapeutic applications, their use has increased dramatically in medical science.

Thus in the present research potent antimicrobial activity of synthesized tungsten nanoparticles were observed. There are several reports (24) on anti-platelet aggregation activities advocated for a new approach, which are recommended for the binding of drug receptors under a laboratory physiological environment. Further, growing appeal for innovations of natural anticoagulants is getting boon due to overwhelming consumer response requiring remedies devoid of undesirable side effects. Therefore, the inhibition of aggregation of platelet formation and anticoagulants using phytoceuticals and nutraceuticals can be promising approach for the prevention of thrombosis. Thus in the present investigation significant antiplatelet activity was observed in synthesised nanoparticles.

The cytotoxic potential of nanoparticles is correlated to physicochemical interface of tungsten atoms stabilized with functional groups of intracellular proteins, along with the nitrogen bases and phosphate groups in DNA. Some scanty reports proved that (25) the nanoparticles having anticancer potential possess ability to reduce expression of signalling proteins, like Akt and Ras, cytokine-based therapies, DNA- or protein based vaccines against particular tumor markers and tyrosine kinase inhibitors which are related to regular antitumor potential (26, 27).

Thus to best of our knowledge this is first comprehensive report on green synthesis of tungsten nanoparticle and their biological activities.

**Conflict of interest**

No conflict of interest was declared by the authors.

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