

## A simple Green Synthesis and Characterization of Selenium Nanoparticles and Evaluation of their in Vitro Anticandidal Activity

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### Abstract

The World Health Organization (WHO) has classified *Candida albicans* as one of the most dangerous pathogenic fungi in the world because of its ongoing development of resistance to anticandidal medicines that are now on the market. The present study addressed this issue by offering a straightforward, one-step, economical, and secure method for the manufacture of novel functionalized anticandidal selenium nanoparticles (Se NPs) against *C. albicans* ATCC10231 using methanolic fruit extract from *Washingtonia robusta* H. Wendl. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the bioactive chemicals in the *W. robusta* H. Wendl extract. Heptasiloxane (44.43%), oleic acid (13.07%), and undecanoic acid, 11-bromo-, methyl ester (4.02%) were among the seven biochemical ingredients found in this methanolic fruit extract. The resulting nanoparticles were characterized using various techniques, including ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), zeta potential analysis, and transmission electron microscopy (TEM). The anticandidal efficacy of the Se NPs was evaluated against *Candida albicans* ATCC10231 using agar well diffusion method, and minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) were determined. Results indicated that the Se NPs exhibited a good anticandidal activity with an inhibition zone  $32 \pm 0.04$  mm, MIC 15  $\mu\text{g/ml}$ , and MFC 15  $\mu\text{g/ml}$  against *C. albicans*. These results indicated to the promising anticandidal action of the green synthesized Se NPs which might be applied in different medicinal and pharmaceutical fields.

**Keywords:** *Washingtonia robusta*, selenium nanoparticles, green synthesis, GC-MS, anticandidal activity.

### Introduction

There are more than 200 species in the genus *Candida*, and 15 of these have been identified from human and animal diseases. The

most common pathogens are *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* (Contaldo *et al.*, 2023) The human digestive tracts microbiota typically includes the opportunistic fungus pathogen *C. albicans*. The typical bacterial flora, on the other hand, developed host defensive systems that enabled

the growth and survival of *Candida* species as commensals. However, a minor change to the host's defenses or biological environment can allow *C. albicans* to grow into a pathogen that can cause infections that are potentially lethal (**Jenkinson & Douglas, 2002**). A balanced combination of energy and metabolites is frequently seen in the microbiota of healthy hosts. The homeostatic balance prevents potentially dangerous bacteria from proliferating uncontrollably. Human diseases are often associated with dysbiosis, or the imbalance of the microbial community (**Li et al., 2022**). Some oral bacteria inhibit the formation of *C. albicans* biofilms, while others encourage it. Furthermore, *Streptococcus oralis* encouraged the development of biofilms and hyphae of *C. albicans* (**Cavalcanti et al., 2017**). According to the World Health Organization (WHO), *C. albicans* is the most hazardous species. Although *C. albicans* is a common microbiome, it can cause opportunistic infections that could be harmful to both humans and animals. The entire body is usually affected by *C. albicans*, including the skin, gastrointestinal tract, oropharynx, lower respiratory tract, and genitourinary system (**Yapar, 2014**). In recent decades, it has continuously been the primary source of invasive infections that can be fatal (**Talapko et al., 2021**). *C. albicans* can be treated with chemical anticandidal medications such as the Azole group, which is divided into two subgroups: imidazole and triazole, however these drugs have serious adverse effects such as hypertriglyceridemia, increased liver enzymes, rash, pedal edema, and hepatotoxicity (**Moudgal & Sobel, 2010**). *Candida* sp. pathogenicity increases due to its capacity to resist accessible antifungal medicines, such as the azole group, which occurs because of target enzyme changes or reduced drug availability to the target (**Contaldo et al., 2023; Moudgal & Sobel, 2010; Yapar, 2014**).

Nanotechnology is a relatively new science that studies and alters the properties of matter at the nanoscale. NPs range in size from 1 to 100 nm and exhibit chemical stability, potential antifungal effects, low toxicity, and low pathogen resistance (**Du et al., 2021; Robles-García et al., 2016a**) Silver, copper, gold, iron oxide, and selenium are important nanomaterials in agriculture, food, the environment, and nanomedicine (**Robles-García et al., 2016b; Saqib, Nazeer, et al.,**

**2022**). NPs may disrupt microbial cell membranes by blocking the activity of Lanosterol 14- $\alpha$ -demethylase, an enzyme involved in ergosterol production and the major sterol element of the fungal cell membrane. Furthermore, NPs may cause reactive oxygen species (ROS), impede spore germination, and regulate protein expression (**Muthuvel et al., 2014; Saqib et al., 2020; Slavin & Bach, 2022**).

Selenium (Se), an essential micronutrient that is also necessary for the upkeep of human physiological systems, is necessary for all mammals (**Yuan et al., 2023**). Furthermore, selenium nanoparticles (Se NPs) exhibit several advantages, including low toxicity, high bioavailability, and degradability, making them safe for clinical use and exceptional in nanomedicine for their antibacterial, antiviral, and anticancer properties (**Alam et al., 2020; Claudia Escobar-Ramirez et al., 2021**). Additionally, because of its anti-inflammatory and antioxidant properties, Se is utilized to treat several pathophysiological conditions, including diabetes, cancer, cardiovascular disease, and neurodegenerative illnesses (**Ferreira et al., 2021**). These NPs can be produced chemically, physically, or biologically; however, the biological technique is more safe and effective than the chemical and physical ways because of the high temperatures, dangerous chemicals, and acidic pH (**Salem & Fouda, 2021**) The low cost, ease of use, safety, enhanced biocompatibility and stability, and non-toxic, high-productivity way of producing NPs make biological synthesis the preferred option (**Fayed et al., 2023**). Living organisms or their natural secondary metabolites, such as proteins from plants (**Ahmad et al., 2023; Al-Zaqri et al., 2021**), fungus (**Saqib, Faryad, et al., 2022**), yeast (**Moghaddam et al., 2017**), algae (**Sharif et al., 2023**), bacteria (**El-Zahed et al., 2023**) and plant as it provided new avenues for the synthesis of NPs and is an ecofriendly, simple, rapid, stable, and cost-effective method. It has advantages, including biocompatibility, scalability, and the medical applicability of synthesizing NPs using the universal solvent, water, as a reducing medium (**Noruzi, 2015**).

Plants offer a sustainable and non-toxic source to produce NPs. Various plant parts, including leaves, fruits, stems, and roots, have been used for green synthesis of NPs due to the

excellent phytochemicals they produce (**Singh et al., 2016**). Natural plant extract synthesis is an inexpensive and environmentally beneficial method that eliminates the need for intermediate base groups. According to published research, certain plants, including *Thlaspi caerulescens*, *Maytenus founieri*, *Arabidopsis helleri*, *Sesbania drummondii*, *Acanthopanax sciadophylloides*, *Clethra barbinervis*, *Brassica juncea*, and *Washingtonia robusta*, accumulate, detoxify, and phytoremediate harmful metals. Because of their enormous potential for environmentally friendly waste removal of contaminants and toxicity, the utilization of these plants in heavy metal elimination from aqueous solutions has drawn a lot of interest (**Agarwal et al., 2017**). Many nanoparticles (NPs), including gold, silver, selenium, zinc oxide, and iron, have been synthesized in a straightforward manner using these green techniques (**Singh et al., 2018**). The current study provided a simple green approach for Se NPs synthesis and investigated their anticandidal potential against *C. albicans* ATCC10231.

## Material and methods

### Sample collection

On October 5, 2023, fresh *W. robusta* fruits were harvested from palm trees in New Damietta City, Damietta Governorate, Egypt, and transported to the Microbiology Laboratory, Faculty of Science, Damietta University for extraction of the phytochemical ingredients. Before drying, any damaged seeds were thrown away and the fruits were thoroughly cleaned to get rid of any debris. After that, the mature, healthy fruits were finely powdered and kept in glass containers at 4°C for physicochemical analysis (**Gomaa, 2019**).

### Plant sample preparation, extraction and gas chromatography-mass spectrometry (GC-MS) analysis

After being smashed with a hammer, the *W. robusta* fruits were mixed and sieved using a 30-mesh stainless steel screen. The leftover residue was mixed one more time until it all went through the screen to ensure a uniform powder. Five grams of this powder were extracted on a shaker for three hours at

room temperature (25°C) after being homogenized for one minute with 40 milliliters of methanol (30%) (**Gomaa, 2019**). Crude extract was dried at 40°C in rotary evaporator and then frozen and lyophilized (EAST Brand WTRE-201D multi-function rotary evaporator total system complete set 2). At the Center for Excellence in Research of Advanced Agricultural Sciences (CERAAS), Damietta University, Egypt, phytochemicals of the resultant extract were identified in accordance with **Elbestawy et al. (2023)** using a GC-MS system (GC-MS QP-2010 Plus, Shimadzu, Kyoto, Japan). The obtained data was gathered utilizing the GC-MS post-run software.

### Green synthesis of selenium nanoparticles

0.007 g of ascorbic acid was mixed with *W. robusta* H. Wendl fruit extract. Then, sodium selenate solution (3 mM) was added to the reaction mixture at a ratio of 1:1 (v/v%) under stirring at 200 rpm until the color changed from pale yellow to red, indicating the formation of Se NPs (**Alagesan & Venugopal, 2019**).

### Characterization of Se NPs

Several methods were used to examine the green-synthesized Se NPs, including Fourier transform infrared spectroscopy (FTIR, FT/IR-4100 type A) and spectrophotometry (Beckman DU-40) at Faculty of Science, Damietta University, Egypt. Zeta potential measurements (Malvern Zetasizer Nano-ZS90, Malvern, UK) and transmission electron microscopy (TEM, JEOL JEM-2100, Japan) were used for additional investigations at the Electron Microscope Unite, Mansoura University, Egypt.

*In vitro* anticandidal activity of fruit extract, Se NPs, sodium selenate and fluconazole using agar well diffusion method

The anticandidal activity of the crude *W. robusta* H. Wendl fruit extract, Se NPs, sodium selenate (bulk) and fluconazole (standard anticandidal drug) were evaluated using the agar well diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (**CLSI, 2017**). *C. albicans* ATCC10231 strain was sub-cultured on yeast extract peptone agar (YEPA) plates and a 10<sup>6</sup> CFU/ml initial inoculum was prepared in yeast extract peptone broth (YEPB) flasks and incubated at 28°C for 24 hours. After the

incubation, the prepared inoculum was added to cooled, molted YEPA flasks, which were subsequently transferred into sterile Petri dishes and let to solidify. 100 µl of each treatment at different concentrations (250, 500, and 1000 µg/ml) was aseptically added to wells done in each plate using a corkborer. Fluconazole was dissolved in dimethyl sulfoxide (DMSO) which was also tested as a positive control. The inhibition zones surrounding the wells were measured in millimeters after 24-hour incubation period at 28°C.

#### Minimal inhibitory concentration (MIC)

The MIC values of Se NPs and fluconazole were determined using the broth dilution method in accordance with CLSI protocol (CLSI, 2012). A 10<sup>6</sup> CFU/ml from *C. albicans* was cultivated on YEPB. Serial solutions (0-100 µg/ml) from Se NP, and fluconazole were prepared and tested. For 24 hours, flasks were incubated at 28 °C. Using an uninoculated broth medium as a blank, the growth of the tested yeast was assessed by measuring the optical density at 600 nm using a Beckman DU-40 UV-Vis spectrophotometer (USA).

#### Minimum fungicidal concentration (MFC)

Each MIC candidal flask was inoculated into YEPA plates using the pour plate method and incubated at 28°C for 24 hours. For each plate containing yeast colony growth the total count was assessed, and MFC values were expressed in colony-forming units per milliliter (CFU/ml) (Mohamed & El-Zahed, 2024).

**Table 1.** Chemical profile of *W. robusta* H. Wendl fruit extract by GC-MS.

Peak	Retention time	Contents%	Compound name	Molecular formula	Molecular weight
1	7.52	1.16	Methanesulfonylacetoneitrile	C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> S	119
2	9.78	2.89	Nitrazepam	C <sub>15</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	281
3	12.33	1.23	4-Benzylidene-3-phenethyl-4H-isoxazol-5-one	C <sub>18</sub> H <sub>15</sub> NO <sub>2</sub>	277
4	16.10	4.02	Undecanoic acid,11-bromo-, methyl ester	C <sub>12</sub> H <sub>23</sub> BrO <sub>2</sub>	278
5	21.07	2.20	Phenytoin	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	252
6	23.33	13.07	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
7	44.20	44.43	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	C <sub>14</sub> H <sub>44</sub> O <sub>6</sub> Si <sub>7</sub>	504

#### Synthesis and characterization of green synthesized Se NPs

The formation of the Se NPs was first indicated

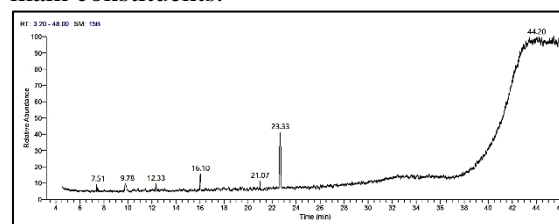
#### Statistical analysis

Software called SPSS version 18 was used to perform statistical analysis on the data. One-way analysis of variance (ANOVA) was used for the analysis, and each experimental value is shown as the mean ± standard deviation (SD). The significance level was set at p<0.05. Every experiment was carried out three times (O'Connor, 2000).

#### Results

##### Chemical composition of *W. robusta* H. Wendl fruit extract using GC-MS analysis

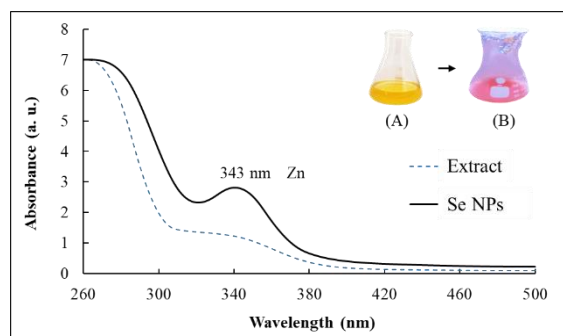
Seven different compounds were found during the GC-MS screening of the crude extract of *W. robusta* H. Wendl fruits (Figure 1 and Table 1). Heptasiloxane (44.43%), oleic acid (13.07%), undecanoic acid,11-bromo-, methyl ester (4.02%), nitrazepam (2.89%), phenytoin (2.20%), 4-benzylidene-3-phenethyl-4H-isoxazol-5-one (1.23%), and methanesulfonylacetoneitrile (1.16%) were the main constituents.



**Figure 1.** GC-MS analysis of the *W. robusta* H. Wendl fruits extract.

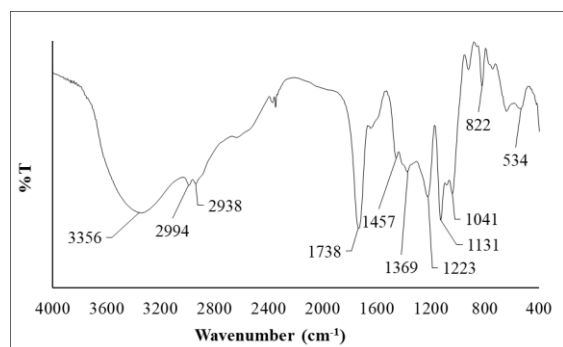
by a color change in the reaction mixture to bright yellow, as illustrated in Figure 2. Within 15 minutes, a red precipitate of Se NPs emerged, confirming their successful green synthesis. Also, an absorption peak at 343 nm

was observed at the UV-Vis spectrum (Figure 2).



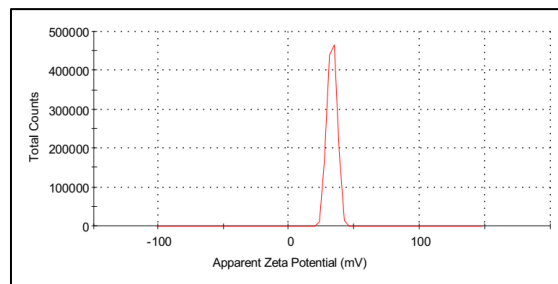
**Figure 2.** Color change and UV-Vis spectroscopy analysis of the Se NPs. (A) Color of the reaction mixture at the beginning of the experiment. (B) Color changes to a red color due to the formation of Se NPs.

To confirm that Se NPs were successfully formed and to detect the presence of capping agents during the synthesis process, FTIR spectroscopy was studied (Figure 3). The O-H stretching vibration of the solvent, methanol, is shown by the peak in the spectrum at  $3356\text{ cm}^{-1}$ . Secondary amines were associated with stretching peaks at  $2994$  and  $2938\text{ cm}^{-1}$ . Vinyl and cis-trisubstituted groups were detected by additional peaks in  $1738$  and  $1636\text{ cm}^{-1}$ , while amine stretching vibrations were detected at  $1457$  and  $1369\text{ cm}^{-1}$ . The C-O was observed at  $1131$  and  $822\text{ cm}^{-1}$ . The stretching vibration of the C-C single bond appeared at  $1041\text{ cm}^{-1}$ . At  $822\text{ cm}^{-1}$ , C-O stretching vibrations appeared. At roughly  $534\text{ cm}^{-1}$ , the Se stretching vibration was detected.



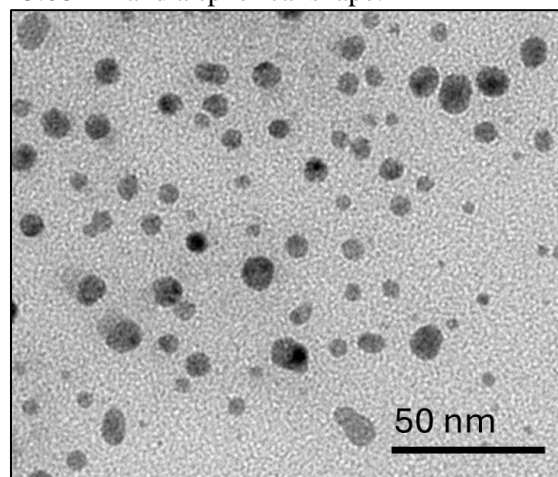
**Figure 3.** FTIR of the green synthesized Se NPs.

The Se NPs had a positive charge of  $-33.4\text{ mV}$ , according to the Zeta potential study (Figure 4).



**Figure 4.** Zeta potential analysis of Se NPs.

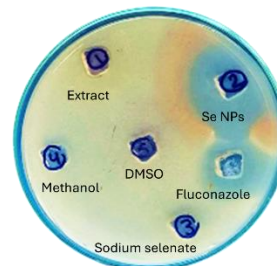
Figure 5 shows TEM pictures of the produced Se NPs. The NPs had an average diameter of  $23.08\text{ nm}$  and a spherical shape.



**Figure 5.** TEM of Se NPs with scale bar =  $50\text{ nm}$ .

#### *In vitro* anticandidal activity

The anticandidal action of the crude extract, green synthesized Se NPs and sodium selenate was assessed against *C. albicans* ATCC10231 compared to standard drug fluconazole, as shown in Figure 6 and Table 2. The green synthesized Se NPs demonstrated significantly higher anticandidal activity against *C. albicans* exhibiting an inhibition zone of  $32\text{ mm}$  compared to fluconazole, exhibiting an inhibition zone of  $19\text{ mm}$ . In contrast, the crude extract displayed the lowest anticandidal activity against *C. albicans*.

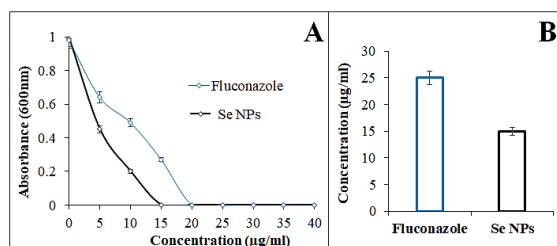


**Figure 6.** Anticandidal activity of Se NPs compared to fluconazole, sodium selenate, and crude extract of *W. robusta* H. Wendl fruits against *C. albicans*.

**Table 2.** The agar well diffusion method of crude extract of *W. robusta* H. Wendl fruits and Se NPs compared to fluconazole and sodium selenate.

Anticandidal agent	Concentration, $\mu\text{g/ml}$	Inhibition zone in mm (mean $\pm$ SD)
Crude extract	250	-ve
	500	$7 \pm 0.14$
	1000	$11 \pm 0.03$
Se NPs	250	$23 \pm 0.14$
	500	$28 \pm 0.06$
	1000	$32 \pm 0.04$
Sodium selenate	250	$7 \pm 0.14$
	500	$10 \pm 0.06$
	1000	$13 \pm 0.06$
Fluconazole	250	$15 \pm 0.03$
	500	$17 \pm 0.03$
	1000	$19 \pm 0$

Se NPs and fluconazole MICs against *C. albicans* were evaluated and shown in Figure 7. At 15  $\mu\text{g/ml}$ , Se NPs totally inhibited *C. albicans*, while it was suppressed by fluconazole at 20  $\mu\text{g/ml}$ . While the MFCs for Se NPs were in line with their MICs, the MFC values for fluconazole were 25  $\mu\text{g/ml}$ .



**Figure 7.** The MICs; A and MFCs; B, of Se NPs compared to fluconazole against *C. albicans*.

## Discussion

One of the most hazardous fungal infections is *C. albicans*, which can cause several illnesses affecting the entire body. The most significant issue is that it can cause resistance to antifungal medications. It can cause major diseases such cutaneous candidiasis, oral and gastrointestinal mucosal candidiasis, vaginal canal candidiasis, and candidiasis, which can cause serious infections depending on the infection site, in both people and animals (Talapko *et al.*, 2021). Numerous investigations have documented examples of azole antifungal resistance in *C. albicans*, even though many antifungal medications, including clotrimazole, econazole, miconazole, terbinafine, fluconazole, ketoconazole, and amphotericin, are extremely irritating and may be fatal (Bossche *et al.*, 2003; Whaley *et al.*, 2017). The goal of this research is to provide a green, simple and cost effective anticandidal

agent with superior activity against *C. albicans*. Methanolic extract of *W. robusta* H. Wendl fruits was selected for the green synthesis of Se NPs. Abutaha *et al.* (2023), found that the methanolic extract of *W. filifera* (Lindl.) H. Wendl. fruit displayed stronger antimicrobial activity compared to other extracts like chloroform, ethyl acetate, and hexane. They reported that the methanolic extract was especially effective against *C. albicans*, achieving an inhibition zone of 30 mm, which exceeded the 20 mm inhibition zone noted for *E. coli*. Furthermore, Adegoke *et al.* (2010) observed that methanolic extracts demonstrated greater antimicrobial activity when compared to chloroform extracts. These findings indicate that methanolic extracts are generally more effective against specific microbial strains than extracts from other solvents.

*W. robusta* H. Wendl fruits were utilized to generate antimicrobial plant extracts that contains many bioactive components as recommended by previous studies (Abutaha *et al.*, 2023; Dewir *et al.*, 2020; Selim *et al.*, 2020). The majority of active compounds found in the GC-MS analysis of *W. robusta* H. Wendl extract were previously reported in the work by Dewir *et al.* (2020). These substances included octasiloxane (1.18%), hydrazine carboxamide (1.67%), decanoic acid (6.47%), n-hexadecanoic acid (6.47%), oleic acid (40.6%), and 1,2,3-propanetriol,1-acetate (3.47%). Different pathogenic fungi, yeast and bacteria were documented to be inhibited by a number of these chemicals, including 1,2,3-propanetriol, 1-acetate, decanoic acid, oleic acid, phenytoin, n-hexadecanoic acid, and octasiloxane (Novak *et al.*, 1961; Saeed *et al.*, 2023; Venkatesh *et al.*, 2014). Additionally, according to Foo *et al.* (2015), n-hexadecanoic acid demonstrated efficacy against *Candida* sp. and *Aspergillus* sp., whereas Muthamil *et al.* (2020) reported the anticandidal action of plant derived oleic acid against *C. albicans* virulence and biofilm formation.

The observed color change during the synthesis process confirms the successful formation of Se NPs. The red color of the produced NPs appeared after 15 minutes of combining a methanolic extract of *W. robusta* H. Wendl fruits with sodium selenate solution. The emergence of a red precipitate verifies their presence and is due to the Surface Plasmon Resonance (SPR) effect. The absorption peaks at 343 nm confirm the optical properties of Se

NPs, which is consistent with previous studies emphasizing the relevance of SPR in NPs characterization, as well as **El-Saadony et al. (2021a)** reported other results ranged between 263 nm and 300 nm. These findings support the efficacy of the synthesis process adopted. The current study recorded a good anticandidal activity for the green synthesized Se NPs against *C. albicans* achieving a  $32 \pm 0.04$  inhibition zone compared to the study of **Fouda et al. (2022)** which reported inhibition zone of  $15.6 \pm 0.6$  against *C. albicans*. **El-Saadony et al. (2021b)** found a MIC and MFC of 55 and 80 µg/mL for LAB-Se NPs against *C. albicans*. **Shakibaie et al. (2015)** generated Se NPs using *Bacillus* species and found a MIC of 70 µg/mL against *C. albicans*. These differences may be attributed to species difference or method of preparation of nanoparticles.

Se NPs are well-known anticandidal agents that primarily target *C. albicans* through oxidative stress. The production of ROS leads to fungal cell wall ruptures, releasing proteins and nucleic acids. ROS also oxidizes critical proteins like glutathione, interrupting major respiratory enzymes and causing bacterial cell death (**Abutaha et al., 2023**). Effective nanometallic compounds, Se NPs cause fungal membranes to become unstable, enhancing cell permeability to NPs. They have shown great potential as all-purpose antibacterials. However, additional engineering is needed to reduce their toxicity to healthy cells and maximize their biological function (**Rofhiwa et al., 2021**). Further studies such as cytotoxicity, antibiofilm and electron microscopy were required to be studied in the future for investigating antimicrobial mechanisms of the prepared NPs as well as their possible applications.

## Conclusions

This study presents a simple and eco-friendly method for green synthesis of Se NPs using methanolic extract of *W. robusta* H. Wendl fruit as both a stabilizing and reducing agent. Se NPs production was validated using a variety of analytical techniques, that showed the successful formation of spherical shaped NPs with average size of  $\approx 23$  nm. The zeta potential and FTIR studies of Se NPs confirmed their long-term stability. Green-synthesized Se NPs had strong anticandidal action against *C.*

*albicans*, with MIC and MFC values ranging from 15 µg/ml compared to fluconazole (20 and 25 µg/ml, respectively). Thus, the prepared Se NPs might be used as a promising anticandidal agent in different pharmaceutical applications.

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## الملخص العربي

### عنوان البحث: التصنيع البسيط والتوصيف لجسيمات السيلينيوم النانومترية وتقييم نشاطها المضاد للكائنات معمليا

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صنفت منظمة الصحة العالمية فطريات الكانديدا كواحدة من أكثر الفطريات المسببة للأمراض خطورة في العالم بسبب تطورها ومقاومتها المستمرة وخاصة للعقاقير المضادة للفطريات الموجودة حاليا بالسوق. تناولت الدراسة الحالية هذا الموضوع من خلال تقديم طريقة مباشرة، بخطوة واحدة، واقتصادية، وأمنة لتصنيع جسيمات السيلينيوم النانومترية ذات الخصائص الفريدة والمضادة للفطريات وخاصة فطريات الكانديدا، تم تصنيع الجسيمات باستخدام المستخلص الميثانولي لثمار واشنطونيا روبوستا. تم استخدام كروماتوغرافيا الغاز المزودة بمطياف الكتلة لدراسة المواد الكيميائية النشطة بيولوجيا في هذا المستخلص وقد أظهرت النتائج أن مركبات هيبثاسيلوكسان (٤٤,٤٣٪) وحمض الأوليك (١٣,٠٧٪) وحمض أونديكانويك، ١١-برومو، ميثيل إستر (٤,٠٢٪) هي الأعلى تواجدا من بين المكونات الكيميائية الحيوية السبعة الموجودة في المستخلص الميثانولي للثمار المستخدمة. تم توصيف الجسيمات النانومترية الناتجة باستخدام تقنيات مختلفة، بما في ذلك التحليل الطيفي فوق البنفسجي المرئي، والتحليل الطيفي بالأشعة تحت الحمراء، وتحليل زيتا، والمجهر الإلكتروني النافذ. تم تقييم فعالية الجسيمات النانومترية ضد فطر الكانديدا باستخدام طريقة انتشار المواد خلال الأجار، والحد الأدنى المثبط، والتركيز المبيد للفطريات. أشارت النتائج إلى أن الجسيمات النانومترية قد أظهرت نشاطاً مضاداً جيداً ضد فطر الكانديدا مصاحبا بمنطقة تثبيط  $32 \pm 0,04$  مم، وقد سجل التركيز المثبط الأدنى ١٥ ميكروجرام / مل، والتركيز الأدنى المبيد للفطريات ١٥ ميكروجرام / مل. ومن هنا تشير هذه النتائج إلى التأثير الواعد لجسيمات السيلينيوم النانومترية المصنعة بطريقة خضراء في مكافحة فطريات الكانديدا، والتي يمكن تطبيقها في مجالات طبية وصيدلانية مختلفة.