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Antimicrobial activities of silver nanoparticles of extra virgin olive oil and sunflower oil against human pathogenic microbes

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Abstract: Silver nanoparticles were synthesized using extra virgin olive oil (*Olea europaea* L.) and sunflower oil (*Helianthus annuus* L.) and characterized by UV–vis spectroscopy, X-ray powder diffraction (XRD) and scanning electron microscopy (SEM). The brown color solution of olive oil nanoparticles (EVOO-NPs) and sunflower oil nanoparticles (SFO-NPs) showed typical absorption at 418 nm and 434 nm respectively. The morphology of extra virgin olive oil was found to be in semi cubic shapes with particle size of 23.45 nm (XRD) and 42.30 nm (SEM) while particle size of (SFO-NPs) had 42.30 nm (XRD) and 46.80 nm (SEM). Antimicrobial activities of crude extra virgin olive oil (EVOO), crude sunflower oil (SFO), synthesized nanoparticle from (EVOO-NPs) and (SFO-NPs) against human pathogenic strains were investigated. Synthesized nanoparticle from each oil showed a potent antimicrobial activity against all tested micro-organisms than crude oil which increased by (81.14% to 174.65 %) and by (111.65% to 192.31 %) than (EVOO) and (SFO) respectively. Both (EVOO-NPs) and (EVOO) had more antimicrobial activities than (SFO-NPs) and (SFO). EVOO (NPs) and SFO (NPs) showed maximum antibacterial activities against *K. pneumoniae*. Therefore (EVOO-NPs) and (SFO-NPs) could be used as safe natural product against multidrug resistant microbes.

Keywords: Antimicrobial activities, *Helianthus annuus*, *Olea europaea*, Silver nanoparticles.

INTRODUCTION

Recently, both gold and silver nanoparticles have generated particular interest as a developmental research tool covering many subjects of sciences. In general, silver nanoparticles (AgNPs), have a number of significant applications in many exclusive areas. It is commonly used as an antifungal and antibacterial agents due to its have good optical characteristics ideal for biochemical imaging and sensing (Ateeq *et al.*, 2015; Khalil *et al.*, 2014; Wijnhoven *et al.*, 2009).

Several methods for Ag and AuNP synthesis have been suggested, such as; thermal decomposition (Navaladian *et al.*, 2007), thermal reduction (Guzmán *et al.*, 2009; Tolaymat *et al.*, 2010), assisted microwave (Sreeram *et al.*, 2008), mediated laser (Zamiri *et al.*, 2011), sodium borohydride (Goswami *et al.*, 2016) and biological reduction (Sastry *et al.*, 2003). In recent years researchers concentrated on discovering nutraceuticals and functional foods as having a significant effect on extending the lifespan and improving health in general. Nutraceuticals have been considered as a diet complement, aid in illness prevention and as a conventional food (Kalra, 2003).

The Mediterranean diet which are rich by olive oil showed correlation with large decrease in coronary heart disease (CHD) mortality, and leaded to the overall improvement several of other phenomena such as in medical and surgical procedures of human (Estruch *et al.*, 2013; Zong *et al.*, 2016). Olive oil is the main source of fat in the Mediterranean area and so why it separated the Mediterranean diet from other diets in their benefits to human health. Olive oil has been used mostly for religious and spiritual purposes in the past since 2000 years and is mentioned in the Holy Koran, Bible, as well as in the works of Homer (Belarbi *et al.*, 2011).

In common, extra virgin olive oil produced completely by mechanical means with no addition of any solvents and at temperatures that do not degrade it (Kiritsakis, 1998; Trichopoulou, 2004). It contains monounsaturated fatty acids, primarily oleic acid, which reduces the level of lipoprotein cholesterol (LDL-C) relative to saturated fat. Olive oil also contains the most common family of bioactive compounds includes carotenoids, sterols, lycopene and hydrophilic phenolics and free fatty acid of about 0.8 grams/100 grams, measured as oleic acid and peroxide index about 20 meq O2/kg oil (Commission,

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1991; Larsen *et al.*, 1999). On the other hands, the popular sunflower (*H. annuus* L.) is a commercially grown plant of the Asteraceae family which offers a variety of dietary and medicinal advantages. It is considered as the world's fourth important source of edible oil following the palm oil, soybean and rapeseed/canola oil (Rosa *et al.*, 2009). Sunflower seeds and sprouts contain valuable amount of antioxidants, antimicrobials, anti-inflammatory, antihypertensive, cardiovascular and wound healing benefits found in their phenolic compounds, polyunsaturated fatty acids, vitamins and flavonoids (Fowler, 2006). It's is always used in ethnomedicine to treat a variety of diseases, including cardiovascular disease, respiratory, laryngeal and respiratory infections, coughs and colds (Bashir *et al.*, 2015). Also, usually used as a vegetable oil, consumed as a roasted or salted snack, dehulled and included as a confectionary nut, and as a meal for livestock and pet (Alagawany *et al.*, 2015).

The presently work investigate the synthesis of silver nanoparticles from extra virgin olive oil (*O. europaea*) and sunflower oil (*H. annuus*) and ascertain their comparative characterization. The crude oil of extra virgin olive oil (*O. europaea*) and sunflower oil (*H. annuus*) and their synthesized nanoparticles were examined against human pathogenic microbes *Viz, Proteus mirabilis*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Candida albicans*, *Micrococcus luteus*, *Staphylococcus aureus* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Biosynthesis of silver nanoparticles

Aqueous solution of silver nitrate (0.5mM) (Sigma-Aldrich, MO, USA) was prepared and mixed with extra virgin olive oil (*O. europaea*) and sunflower oil (*H. annuus* L.) at a ratio of 9:1 and heated at 70°C for 1 minutes (Pirtarighat *et al.*, 2019). The solution turned from yellowish to brown and to dark brown for silver nanoparticles formations (Ingle *et al.*, 2009). The Erlenmeyer flasks were incubated at 4°C for further works. All stages of the experiment were done in three replicates.

Characterization of silver nanoparticles

Absorption spectrum was taken at 300–540 nm with a UV-vis-spectrophotometer (HITACHI, Model U-2800 spectrophotometer) to check NPs formation from two oils. The de-ionized water has been used as the blank. Crystallinity was proved at room temperature by X-ray diffraction technique (XRD) using Shimadzu LabX-XRD-6000 diffractometer with CuKα (λ¼1.5406 Å) radiation and secondary monochromator attached with Shimadzu software with the pdf-2 library for the analysis of XRD data. The nanocrystalline size has been calculated by applying the equation of Debye–Scherrer formula: (D= kλ/βcosθ, (D is particle diameter size, k is a constant = 1, λ is wavelength of X-ray source (0.1541 nm), β is the full width at half maximum (FWHM) and θ is the diffraction angle corresponding to the lattice plane (111)). The samples from each oil types of formed Silver nanoparticles were placed on double-sided adhesive tape specimen stubs and studied via SEM (JSM-7500 F; JOEL-Japan).

Pathogenic strains

Seven pathogenic human pathogenic microbes namely, *P. mirabilis*, *S. flexneri*, *P. aeruginosa*, *C. albicans*, *M. luteus*, *S. aureus* and *K. pneumoniae* were first grown in nutrient broth (NB) and incubated for 48 h for further use. Isolates were obtained from departments of Biology and Microbiology, King Khalid University, Saudi Arabia.

Antimicrobial activity by well diffusion method

Crude oil of extra virgin olive (EVOO), synthesized nanoparticle of extra virgin olive oil (EVOO-NPs), crude sunflower oil (SFO) and synthesized nanoparticle of sunflower oil (SFO-NPs) were tested for their antimicrobial activity by a test of well diffusion against human pathogenic microbes. Every strain was uniformly swabbed onto the individual plates using sterile cotton swab and in each plate wells of 6 mm size were made on Muller – Hinton agar plates using gel puncture. Using micropipette, 100ul from each sample either from crude oil or nanoparticles solution were poured into 3 wells on each plate. Dimethyl sulfide (DMS) 10% was applied as a negative control and 30 mcg Cefoxitin as a positive control. All plates were incubated at 35°C for 48 h, then resulted inhibition zones were observed and measured in millimeters (Jena *et al.*, 2016).

STATISTICAL ANALYSIS

Statistical analysis was performed for three triplicates using one-way ANOVA (Dunnett's multiple test).

RESULT

Antimicrobial activity

Antimicrobial inhibition activities of extra virgin olive oil (EVOO), extra virgin olive oil nanoparticles (EVOO-NPs), sunflower oil (SFO) and sunflower oil nanoparticles (SFO-NPs) against pathogenic microbes are shown in table 1. It was found that crude oil of extra virgin olive oil, sunflower oil and their synthesized nanoparticles exhibited antimicrobial activities with various potency against all tested microbes. For all antimicrobial inhibition activities, it was evident that the synthesized nanoparticle showed a potent activity against all tested micro-organisms than the crude oil itself recording increments by (81.14% to 174.65 %) than (EVOO) and by (111.65% to 192.31 %) than (SFO). In addition, each of (EVOO-NPs) and (EVOO) had a potent antimicrobial activities than (SFO-NPs) and (SFO) by (6.87% to 27.69%) and by (2.63 % to 10.75%) respectively. The

negative control did not show any antimicrobial activities, while the positive control showed halo indicative zone in the range between (21.93±0.26 mm to 36.83±2.08 mm). However, both NPs from natural oils either from (EVOO) or (SFO) showed more potent activities than Cefoxitin by (5.89% to 38.35%) and by (3.18% to 33.68%) respectively. EVOO (NPs) showed the maximum antibacterial activities against *K. pneumoniae* with inhibition killing activities of (39.00±3.51 mm) followed by *P. aeruginosa* with inhibition zone of (30.90±2.15 mm). *S. aureus*, *M. luteus*, *C. albican*, *S. flexneri* and *P. mirabilis* affected by the EVOO (NPs) in the range between (26.90±3.50 mm to 29.63±2.15 mm). The antimicrobial trends obtained from (SFO-NPs) had the maximum activities against *K. pneumoniae* with inhibition zone of (38.90±2.51 mm) followed by *P. aeruginosa*, *S. aureus*, *M. luteus*, *C. albican* and *P. mirabilis* having inhbition zone between (28.69±2.99 mm to 26.10±2.50 mm). *S. flexneri* had the lowest susceptibility from SFO (NPs) having inhibition zone of (26.10±2.50 mm).

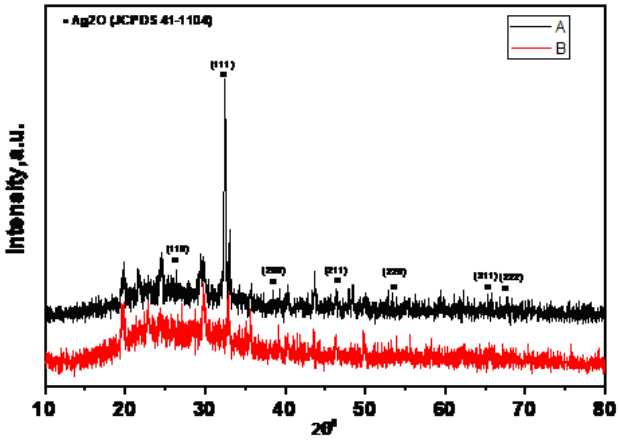

Fig. 1: X-ray diffraction (XRD) pattern of NPs powder of Extra Virgin Olive oil (EVOO) (A); and Sunflower Oil (SFO) (B).

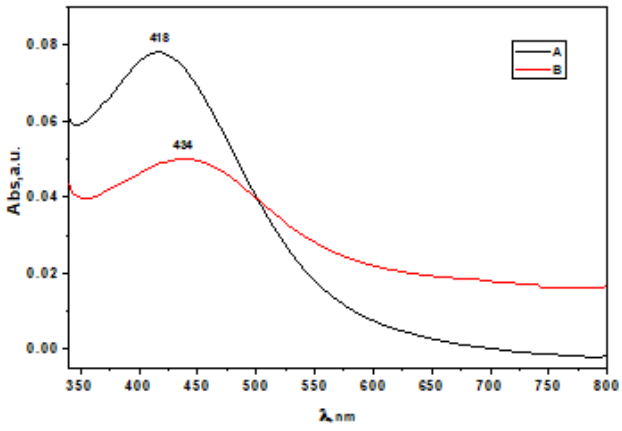

Fig. 2: UV absorbance spectra of Extra Virgin Olive oil (EVOO) (A); and Sunflower Oil (SFO) (B).

X-ray diffraction (XRD)

Fig.1 represents the X-ray diffraction spectra and Miller indices (hkl) of (EVOO-NPs) and (SFO-NPs). The pattern showed diffraction peaks at 26.940, 32.270, 38.320, 46.340, 54.920, 65.460 and 68.480 which corresponds to (110), (111), (200), (211), (220), (311) and (222) planes of face- centered cubic silver, respectively. These peaks corroborate with the standard Ag_2O (JCPDS 41-1104). The other peaks observed may be due to the difference between the nature compositions of the two oils. The tabulated data (table 2) indicate strongly that for (EVOO-NPs) and (SFO-NPs) has an effect on the average crystallite size for Ag NPs.

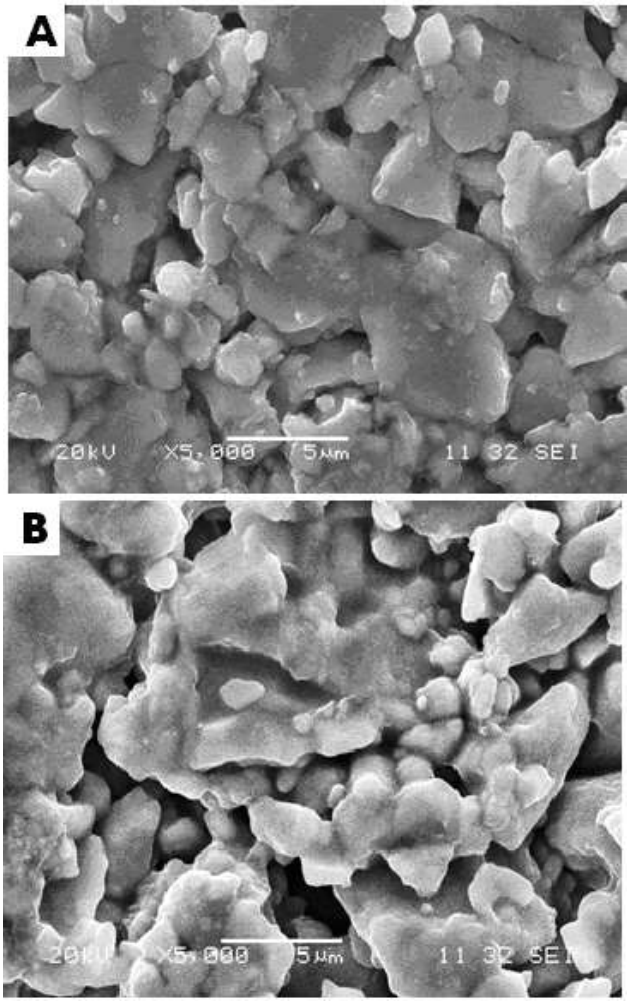

Fig. 3: SEM images of biosynthesized EVOO (NPs) (A) and SFO (NPs) (B).

UV–visible

Fig. 2. shows the UV–visible spectra of silver nanoparticle formation using constant concentrations of 9 ml of AgNO3 (0.5mM) added to 1ml of each oil that heated at 70°C for 1 minutes. The color of the solutions changed from yellowish to brown and to dark brown depending on the nature of two oils types due to the excitation of surface Plasmon vibration of silver

Table 1: Antimicrobial activities of EVOO (NPs), EVOO, SFO (NPs) and SFO in mm

	Pathogen						
Sample	*K. pneumoniae*	*S. aureus*	*M. luteus*	*C. albicans*	*P. aeruginosa*	*S. flexneri*	*P. mirabilis*
EVOO (NPs)	39.00±3.51	29.63±2.15	28.69±2.99	29.33±3.51	30.90±2.15	28.0** ±0.00	26.90±3.50
EVOO	14.20* ±1.53	13.58 ±2.5	13.54* ±1.54	15.64±1.57	13.57±3.52	14.36** ±0.5	14.85* ±1.59
SFO (NPs)	38.90±2.51	28.34±2.15	28.69±2.99	28.34±2.81	27.90* ±1.15	25.00* ±1.00	26.10±2.50
SFO	13.00* ±1.43	11.00* ±1.63	12.67* ±1.08	13.39±2.51	11.20* ±1.10	11.38* ±1.14	11.63** ±0.39
PC	36.83±2.08	22.00±2.01	24.52* ±1.53	21.20* ±1.60	23.9* ±1.40	21.93** ±0.26	23.91±1.48
NIZ	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ

NIZ, no inhibition activities; PC, Positive control, (Cefoxitin- 30 mcg). Significant differences (*$P \leq 0.05$;** $P \leq 0.01$), between treatments ± SD of the mean for n = 3.

Table 2: Grain size (nm) using (XRD and SEM) and absorption band (nm) of EVOO (NPs), and SFO (NPs).

Sample	Grain size (nm) (XRD)	Grain size (nm) (SEM)	Absorption band (nm) UV–visible
EVOO (NPs)	23.45	42.30	418
SFO (NPs)	22.43	46.80	434

nanoparticles. It can be seen that the surface plasmon resonance (SPR) of AgNPs is 418 nm and 434 nm for Extra Virgin Olive oil (EVOO) and Sunflower Oil (SFO) respectively (Goodarzi *et al.*, 2014; Sosa *et al.*, 2003).

SEM analysis

Scanning electron microscopy (SEM) analysis is performed for studying the surface morphology and shapes of silver nanoparticles (fig. 3). It is observed that the silver nanoparticles are more or less semi clearly cubic in shape for EVOO (NPs) and in agglomeration shape than SFO (NPs). This indicates the formation of Ag NPs by EVOO (NPs) differs from SFO (NPs) which supported by the intensity in XRD pattern (fig. 1) and the area under peak in surface plasmon resonance region (fig. 2). The average particle size was 42.3 nm and 46.8 nm for EVOO (NPs) and SFO (NPs), respectively (table 2).

The detailed study on silver nanomaterial biosynthesis by Extra Virgin Olive oil (EVOO) and Sunflower Oil (SFO) were reported in this research. The aqueous silver ions were reduced to silver nanoparticles when added to Extra Virgin Olive oil (EVOO) and Sunflower Oil (SFO). It was observed that the color of the solution turned from yellowish to brown and then to dark brown after 1 minute for Extra Virgin Olive oil (EVOO) and the time increased little to be brown then to dark brown for Sunflower Oil (SFO) which indicated the formation of silver nanoparticles.

The formation and stability of the reduced silver nanoparticles for Extra Virgin Olive oil (EVOO) and Sunflower Oil (SFO) in the colloidal solution was monitored by UV-vis spectrophotometer analysis. The UV-vis spectra showed maximum absorbance at 418 nm for Extra Virgin Olive oil NPs whereas for Sunflower Oil NPs were noticed at 434 nm corresponding to the surface plasmon resonance of silver nanoparticles.

DISCUSSION

Characterization of formed AgNPs from both types of oils includes various parameters. The sharpness and increase in intensity of spectra means that AgNPs extracted by (EVOO) had more crystallinity than AgNPs extracted by SFO which might be due to the enhanced density of decorated crystalline AgNPs (Riaz *et al.*, 2015; Wani *et al.*, 2011).

Previous report indicated that absorbance is distinguished to the silver particles at around 430 nm (Vilchis-Nestor *et al.*, 2008). The appearance of intensity peak in the region of 418 nm and 434 may be depends on the particle size and the shape of each oil either Extra Virgin Olive oil or Sunflower Oil (Jain *et al.*, 2006; Kelly *et al.*, 2003).

Color solution showed the formation of silver nanoparticles in varying times period whereas the Extra Virgin Olive oil (EVOO) had less time than the Sunflower Oil (SFO). In addition a well dispersed nanoparticles could be seen in the oil of Extra Virgin Olive oil (EVOO) treated with silver nitrate than Sunflower Oil (SFO) due to particles agglomeration results (Narmadha *et al.*, 2013).

The absorption intensity increased in their ratio by applying the Extra Virgin Olive oil (EVOO) indicating the complete reduction of silver ions, which may be due to the decrease in the number of AgNPs because of aggregation (Kumar *et al.*, 2014; Moores and Goettmann, 2006; Munir *et al.*, 2017). It is observed that the silver nanoparticles from (EVOO) are more or less semi cubic

in shape, suggesting that this specific shapes might play an important role in various biological activities (Millstone *et al.*, 2005; Xie *et al.*, 2017). However, (Ahmed *et al.*, 2018) prepared silver nanoparticles using olive oil (O-AgNPs) as capping and reducing agents found that their morphology being a spherical in nature with a typical absorption at 430 nm and the size of O-AgNPs in the range between 35 to 65 nm. This interprets that natures of the synthesized sliver nanoparticles vary greatly depends on the oil nature of the plant even at the level of the same species. Herein, we considered the effects of deviations from the cubic shape to spherical shape probably due chemical nature of the oils that effect on the plasmonic response of silver particles. The same species can vary in the types of compounds that they are producing and/or the relative quantities of shared compounds and the presence or absence of specific compounds, or differences in compound ratios. These differences may be as a results of many factors like geographic range of a species, the evolutionary processes, phenotypic variation, ages, soils, prevailing environmental conditions, harvest time, extraction methods, varieties, etc (Agrawal, 2011; Deng *et al.*, 2012; Fang *et al.*, 2011; Kurihara *et al.*, 2003; Liu *et al.*, 2016b; Nazzaro *et al.*, 2019; Poelman *et al.*, 2008; van Dam and Heil, 2011; Xi *et al.*, 2014). Recent studies had reported that light conditions, genotypes, and fertilization levels significantly affect chemicals in plants (Deng *et al.*, 2012; Liu *et al.*, 2016a). In addition various oils under the same conditions showed various SPR peak and shapes of the particles as in Sacha inchi (*Plukenetia volubilis* L.) oil that revealed two main SPR peaks (at 380 and 480 nm) often observed for cubical/square shape AgNPs. However, coconut oil revealed single SPR peak at 410 nm and spherical shape using TEM images (Kumar *et al.*, 2014; Meena Kumari and Philip, 2013).

The enhanced antimicrobial effects of synthesized silver nanoparticles were characterized from Extra Virgin Olive oil (EVOO) and the Sunflower Oil (SFO) against human pathogenic microbes. The results showed that silver nanoparticle gained from (EVOO) and Sunflower Oil (SFO) had more inhibitory efficacy than crude oils against *P. mirabilis*, *S. flexneri*, *P. aeruginosa*, *C. albicans*, *M. luteus*, *S. aureus* and *K. pneumoniae* with various diameter of inhibition zone. This is probably due that synthesized nanoparticles had the ability to damage the cell wall or/and damage the DNA chemical structure by interacting with phosphorus and sulfur residues leading microbial cell death (Kazachenko *et al.*, 2000; Rai *et al.*, 2009). Differences in the nature of the size particles between the Extra Virgin Olive oil (EVOO) and the Sunflower Oil (SFO) may be lead to the differences between their antimicrobial activities in agreement with previous research (Gliga *et al.*, 2014; Su *et al.*, 2017; Zafar *et al.*, 2016). It was found that the factor affecting bactericidal properties of silver nanoparticles is their shape, size, surface area, whereas the small size of silver nanoparticles has high surface reactivity. In addition, (Abbaszadegan *et al.*, 2015) reported that the bactericidal abilities of silver nanoparticles are correlated with the electrostatic reaction between the negative charge of the bacterial cell wall and the positive charge of metal ions. In future it is worth to do more details study about Extra Virgin Olive oil (EVOO) to characterize and identify specific chemical and generate NPs against specific human pathogenic microbes.

CONCLUSION

In conclusion, reported features indicate that both Extra Virgin Olive oil (EVOO) and Oil (SFO) could be used as safe natural product to synthesis silver nanoparticles having potent antimicrobial characters.

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