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Al-Hamdani & Ahamed

المجلة العراقية لبحوث السوق وحماية المستهلك

PREPARATION OF A COMBINATION OF NANO- MEDICINAL PLANTS AS ANTIOXIDANTS AND MICROORGANISMS

Hamdia, M. S. Al-Hamdani [1], *Sundus H. Ahamed* [2]
[1] Assistant Professor PhD. Market Research and consumer protection center, University. of Baghdad, Baghdad, Iraq, cioffi16@yahoo.com
[2] Assistant Professor PhD. Biological Department, Al-Mustansiriya University, Baghdad, Iraq. sundusahmed@yahoo.com

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ABSTRACT

The controversy is currently revolving around industrial additives, including antioxidants, their negative effects on consumer health and the emergence of various and various diseases, which led scientists and researchers to intensify most studies on natural antioxidants and their synthesis from medicinal plants mentioned in ancient medicine and in divine books as potential antioxidants of increasing importance. Therefore, this study was designed to synthesize silver nitrate particles from plant leaf extracts (Figs, Olives, and Moringa) and study their effect on bacterial inhibition of each of the undesirable Coliform bacteria (E-Coli,- gram) and the streptococcus bacteria of (Staphylococcus aureus + Gram) and antioxidants after analysis of phytochemicals and essential compounds of these plants. The results showed that it contained a high percentage of phenols and total flavonoids, followed by coumarin, then it contained good amounts of alkaloids, tannins, saponins, resins, terpenes and steroids. The study also showed a high and significant inhibition of the plant mixture nano extract on coliform bacteria, as well as streptococcus bacteria, in addition to its effectiveness as an antioxidant compared to the synthetic antioxidant Butylated Hydroxy Toluene (BHT) and the extract of the natural combination. The results of this study proved that the leaves of the plants blend (Fig, Olive and Moringa) are an excellent natural source of anti-bacterial and antioxidant effects that interfere with their effects on public health. Therefore, this botanical mixture, as mentioned in the Great Book of God (Figs, Olives, and Sennin phase) has great benefits and a promising future in several areas, including nutritional supplements and food additives for various food products instead of industrial oxidation additives and their known side effects, in addition to that this mixture has a promising future. Also in the manufacture of fast-curing Nano medicines in direct contact with pathogens. .

Keyword: Plant blend (Fig, Olive and Moringa), antibacterial, antioxidant.

تحضير توليفة من النباتات الطبية نانويا كمواد مضادة للأكسدة وللأحياء المجهرية

حمدية محمد شهوان الحمداني [1]، *سندس حميد احمد* [2]
1. الاستاذ المساعد الدكتور، مركز بحوث السوق وحماية المستهلك، جامعة بغداد، بغداد، العراق. cioffi16@yahoo.com
2. الاستاذ المساعد الدكتور، كلية العلوم، قسم علوم الحياة، الجامعة المستنصرية، بغداد، العراق. sundusahmed@yahoo.com

الخلاصة

يدور الجدل حاليًا حول المضافات الصناعية ومنها مضادات الأكسدة وآثارها السلبية على صحة المستهلك وظهور أمراض مختلفة ومتنوعة، مما قاد العلماء والباحثين إلى تكثيف معظم الدراسات حول مضادات الأكسدة الطبيعية وتوليفها من النباتات الطبية المذكورة في الطب القديم وفي الكتب الإلهية كمضادات أكسدة محتملة ذات أهمية متزايدة. لذلك صممت هذه الدراسة لتصنيع جزيئات نترات الفضة من مستخلص أوراق النبات لتوليفة (التين، الزيتون والمورينجا) ودراسة مدى تأثيرها على التثبيط البكتيري لكل من البكتريا القولونية غير المستحبة لصبغة كرام (E-Coli, - gram) والبكتريا

العقدية المستجبة لصبغة كرام (Staphylococcus aureus + gram) ومضادات الأكسدة بعد تحليل المركبات الكيميائية الحيوية والمركبات الأساسية لهذه النباتات. أظهرت النتائج احتوائها على نسبة عالية من الفينولات والفلافونيدات الكلية، يليها الكومارين، ثم احتوت على كميات جيدة من القلويدات، والتانينات، الصابونين، الراتنجات، التربينات والستيرويدات. كما أظهرت الدراسة تثبيط عالي ومعنوي للمستخلص النانوي للمزيج النباتي للبكتيريا القولونية، وكذلك البكتيريا العقدية، بالإضافة إلى أنها أثبتت فعاليتها كمضادات للأكسدة مقارنة بمضادات الأكسدة الصناعية BHT Butylated hydroxytoluene ومستخلص التوليفة الطبيعية. وقد أثبتت نتائج هذه الدراسة أن أوراق النبات لكل من (التين، الزيتون والمورنيجا) هي مصدر طبيعي ممتازة للتأثيرات المضادة للبكتيريا ومضادات الأكسدة التي تتداخل مع تأثيراتها على الصحة العامة. لذلك فإن هذا المزيج النباتي كما ورد في كتاب الله العظيم (التين والزيتون وطور سينين) له فوائد عظيمة ومستقبل واعد في عدة مجالات منها المكملات الغذائية والمضافات الغذائية لمختلف المنتجات الغذائية بدلاً من مضافات الأكسدة الصناعية وآثارها الجانبية المعروفة، بالإضافة إلى أن هذا المزيج له مستقبل واعد أيضًا في تصنيع الأدوية النانوية السريعة الشفاء للتلامس المباشر مع مسببات الأمراض.

الكلمات المفتاحية: تركيبة نباتية نانوية (التين، الزيتون والمورنيجا)، مضاد البكتريا، مضادات الأكسدة.

INTRODUCTION

Nature is an essential source of substances necessary for human needs. Scientists used most of the medicinal materials and pharmaceutical preparations to extract effective compounds from various medicinal plants known by our ancestors and used to combat various diseases or to prepare medicines from natural sources **(Shahinuzzaman *et al.,* 2020)**. Since ancient times, there have been common uses and practices and systems in Indian and Arab medicine and in most East Asian countries and cities bordering the Mediterranean for natural products as mixtures (**Suleiman, 2014**). These natural products provide pharmacologically active compounds that work perfectly especially as anti-microbial, anti-parasitic and anti-cancer (**Mina *et al.,* 2020**).Scientific studies have proven that the oxidative stress that occurs to human is due to many internal or external pathological factors such as parasitic infections that disrupt the oxidation / antioxidant balance, and scientifically proven positive and close link between antioxidants and the body's defense system with nutrition (**Harvey *et al.,* 2015)**. Currently health awareness has increased about the effects of using industrial and widely used antioxidants in food and medicine and their negative effects on public health. Thus, research and investigation increased to extract antioxidants from various medicinal plants, as our ancestors used them since time immemorial, and they are receiving increasing attention because they contain important polyphenols and flavonoids compounds for the human body as antioxidants and free radical remover that occurs to humans from various sources (**Shahinuzzaman *et al.,* 2019**). Among these plants are fig leaves, manga, and olives they are considered a lot because they are natural sources of polyphenols and their bioactive metabolites. Moringa, *Moringa Oliveira L*., olive *Olea europaea L*. has historically belonged to the Moringaceae family and has been used as a herbal medicine in Indians, Pakistan, Philippines, Thailand, Africa and Iran as a herbal medicine, as well as that it has been used as a high nutritional supplement and various medicinal substances (**Alaqad & Saleh, 2016**). Figs belong to the genus Ficus and are among the most desirable fruits that are eaten fresh or dried and are mentioned in God's holy book (the Qur'an). Figs are a complete food and a plentiful source of sugars, carbohydrates, vitamins, minerals, organic acids, and phenolic compounds (**Idrus *et al.,* 2018**). Olives are also one of the most common fruit crops in the world and throughout the Middle East (**Kheirandish *et al.,* 2017**). Previous extensive studies have shown the importance of using olives and its various parts in folk medicine to treat some diseases (**Kheirandesh *et al.,* 2017**), as well as as an anti-diabetic, anti-cancer, anti-inflammatory, antihypertensive, antimicrobial, and parasitic effects **(Hashemi *et al.,* 2015)**. The current trend is to synthesize silver nanoparticles (Ag-NPs) using various fungi, bacteria or plant extracts to discover alternatives. An approach in treating various diseases or for the manufacture of various industrial and food products. There are many reasons and interest in green biosynthesis methods for (Ag-NPs). The reasons are many, because they are simple, it is effective in relation to its low cost, large volume availability, harmless and environmentally friendly **(Zhang *et al.*, 2013**). Extensive studies have used the biosynthesis of Ag-NPs using ethanol extract from different botanical leaves such as *Cardiospermum halicacabum L.* (**Ibrahim, 2015; Hamdia & Sundus, 2019)**, *Impatiens balsamina L.* leaves **(Khor *et al.*, 2018),** Olive tree, *Olea europaea L.* (**Jabri *et al.*, 2017)**, Fig (**Yeganeh & Hoseini, 2017**) as antimicrobial, anti-parasitic, anti-inflammatory, and immune-stabilizing effects, the reason is due to the high content flavonoids and polyphenols that have proven powerful antioxidant effects (**Saedi *et al.*, 2016; Kheirandish, *et al.*, 2017)**. Therefore, the study aimed to test the efficacy of bio-synthesis silver nanoparticles using a leave mixture of Fig *Ficus carica Linn*, Olive *Olea europaea L.* and Moringa *Moringa Oliveira L.* against Gram-negative and positive pathogenic microbes and as antioxidants.

MATERIALS AND METHODS

Plant material and extract preparation

The leaves of blend (Fig, Olive and Moringa, FOM) plants were collected from one of the private orchards located on the outskirts of Baghdad. Then only the leaves were picked, without the stems, and equal weights were taken from each of the three aforementioned plants, and the surface was cleaned with running tap water to remove dust and other contaminated organic contents. By double distilled water, the leaves washed again and were dried using the conventional method after rolling the leaves in stainless steel trays in the shade and under the ceiling fan for five days at Market Research & Consumer Protection Center laboratory at room temperature (20-25 °C). Then dried blend was grounded in a wiley mill to pass through (1 mm) sieve. Then about (10 grams) of the dried, grounded leaves were taken in a beaker contains (100 ml) of distilled water and incubated for 10 minutes at 60 °C, the extract was cooled and filtered with Whatman No.1 filter paper. The resulting extract (FOM) was transferred directly to the laboratory for the synthesis and assay of Ag-NPs synthesized as described by **(Ahmed *et al.*, 2016; Hamdia & Sundus, 2019**) and to determine the antibacterial and antioxidant according to (**Altemimi *et al.*, 2017; Shahinuzzaman *et al.*, 2019).**

Phytochemical screening

The extract of ethanol and hexane of blend (FOM) prepared for phytochemical detection to reveal the various biological components of the plant such as pH, Tannins, Carbohydrates, Glycosides, Phenols, Resins, Flavonoids, Saponins, Alkaloids, Protein, Camarines, Trepans and Steroid was done using standard protocols and according to (**Aman & Gupta, 2020)**.

Green synthesis of silver nanoparticles

The green silver nanoparticles were made by bio-reduction of Ag^+ using plant-blend extracts of each of the leaves (Fig, Olive, and Moringa FOM), and the process was done by adding about (5 mL) of the pre-prepared extract drop by drop to an aqueous solution of $AgNO_3$ (50 mL, 0.1 M) and stirred at 45-50 ° C for 30 minutes. Then, the mixed solution was subjected to ultra-sonication over the solution for 3 hours. The complete synthesis and formation of Ag-NPs was confirmed by changing the colorless silver nitrate solution to a dark brown one, after which the residual $AgNO_3$ was removed by hemodialysis against deionized water at 4 °C. The stability of Ag-NPs of NFOM was also examined by observing the color of the solution after 20, 40, 50 and 60 days of storage at 4 °C. The experiment followed by (**Ahmed *et al.*, 2016**).

Characterization of the biosynthesized SNPs

The different properties of biosynthetic nanoparticles were demonstrated with (Fig, Olive, and Moringa FOM) extract, and these measurements were confirmed in laboratory-University of Kashan/Iran through an agent CAC company in Baghdad city, Iraq.

UV-Visible absorption spectrophotometer

UV-Visible absorption spectrophotometer with an accuracy of (1 nanometer) and a measurement ranging between (200 and 800 nanometers) it was used. (1 ml) of plant extract was withdrawn and placed in a test tube, after which the absorbance was read at different wavelengths for analysis at room temperature. The reaction and absorbance were monitored at different time intervals and using different concentration (1, 2, 3, 4 and 5 mM) of the synthesized silver nitrate nanoparticles with leaf extract (1-5 ml). Dynamic light disintegration is used (Spectro scatter 201) for UV-Spectroscopy to determine the average size of synthesized silver nanoparticles. This result came a close with **(Alaqad & Saleh, 2016)**.

X-Ray Diffraction (XRD) characterization

The XRD characterization method was used to determine the crystallinity of green synthesized AgNPs. The X-ray diffraction device (XRD, 6000- Shimadzu X-ray diffraction meter) was used to examine the purity and quality of the prepared silver nanoparticles and to a certain phases of the crystal structure of silver nanoparticles (**Sudad *et al.*, 2020)** by suspending the nanoparticles and applying the suspension in the form of droplets on the XRD plate until a well-flat layer of particles is obtained. The solution was dropped and dried on a glass substrate (Philips PW) and Cu-Kα crystal radiation was irradiated at a wavelength ($\lambda = 1.54$ A°), and the scan angle (2θ) was varied from (20^o-70^o) degrees at (20^o min^{-1}). The diffraction signal readings were recorded and processed using Diffraction with the addition of particle size measurement from the length of the XRD planes and the estimation was used to measure the crystal size using Scherrer equation recollection according to **(Alaqad & Saleh, 2016)**.

Electron microscope

High-resolution electron microscopy was used to determine the morphology of the nanoparticles. The scanning electron microscope includes (SEM) (TEM) of the synthesized silver nanoparticles AgNPs with the plant extract of the combination, which we can visualize when the electron beam hits nanoparticles (**Noah, 2019**). Then the information is described qualitatively and quantitative information regarding size, shape, Size distribution and dry diameter distribution. The device (TEM) works in accelerated voltage 120 kV at different magnifications, as indicated on the SEM and TEM images **(Sudad *et al.*, 2020**). Photoluminescence was examined and evaluated using a fluorescent eclipse spectrophotometer (Agilent techniques).

Atomic force microscopy (AFM)

AFM is also used to analyze volume, surface morphology, structural and physical mechanical properties by silicon probe saturated with phosphorous (**Noah, 2019)**. The AgNPs sample was prepared by dissolving it in water or ethanol and a drop was placed on a silicon substrate, then, it was left for a period of minutes until it dried and then analyzed by AFM technology using a probe according to **(Jain *et al.*, 2021).**

FTIR characterization

The FTIR technique is used to analyze and detect the elements, chemical structure, chemical bonds, effective groups and bond arrangement in the prepared nanoparticles. An infrared analysis of iron Nano forms was performed with FTIR-8400S, SHIMATZW-FTIR spectroscopy with a wavelength ranging between (500 - 4000) cm^{-1} (**Noah, 2019**). The use of FTIR is to find the particles and their functional groups present in the prepared nanoparticles AgNPs.

Evaluation of antimicrobial assay

The standard disc diffusion method was used on human pathogenic bacteria *Escherichia coli and staphylococcus*. It was used Maconkey broth (HiMedia) to culture the bacteria and incubate at 37 °C / 24 hours after which the fresh growing bacteria were taken and spread on McConkey agar plates to grow the bacteria. After that, sterile paper saturated with nanoparticle plant extract with a diameter of (5 mm) was placed, and the control sample was made with double distilled water, and another sample was made using only the blend extract. The dishes were placed together and simultaneously in an incubator and incubated at 37 °C for 24 hours. After that, the antibacterial activity was measured based on the inhibition of the area around the disc for all treatments (**Hamdia & Sundus, 2019).**

Free radical scavenging assay

The antibacterial activity was determined by Spectrophotometry by monitoring from disappearance of 1,1-diphenyl-2-picrylhydrazyl, DPPH) at 520 nm, according to the described procedure with some modifications (**Ahmed *et al.*, 2016**). First, the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) solutions was prepared by dissolving (2 mg) of this indicator in (100 ml) of methanol. Secondly, the DPPH solution (3 ml) was added to (1 ml) of blend leaf extract and mixed gently according to (**Altemimi, 2017**). The control sample was prepared by mixing (1 ml) of methanol with (3 ml) of the prepared DPPH solution. The synthetic antioxidant Butylated Hydroxy Toluene (BHT) was used as a model for comparison. All mixture were incubated in a dark place for 30 min. Then after that it was measured and recorded the absorbance at 517 nm for both samples using a spectrophotometer. Finally, the inhibition of DPPH activity was calculated using the following equation:

% Inhibition of DPPH activity= $(Ac - As/Ac) \times 100$

Ac: absorbance of the control As: absorbance of the sample

STATISTICAL ANALYSIS

The Statistical Analysis System- **SAS (2012**) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

RESULTS AND DISCUSSION

The chemical composition of each plant and the plant blend (FOM).

The chemical composition of the plant blend and each component of the blend are represented in the (Table, 1). The results of the study showed the high and significant ($P \leq 0.05$) content of ash, fiber and carbohydrates for the plant mixture, as well as containing significant amounts of protein and fat as shown in Table, 1. These results are identical to what was found by (**Rania *et al.*, 2019**).

Table (1): Chemical composition of the dried leaves of the botanical blend and the production ratio of their extracts.

Compenents%	Moringa	Olive	Fig	Plant blend (FOM)	LSD value
Yield	14.50	13.00	13.67	-	2.58 *
Moisture	0.90	0.85	0.92	0.90	0.207 NS
Ash	4.65	5.68	5.80	5.28	0.693 *
Protein	2.85	2.65	2.56	1.50	0.622 *
Fat	2.5	3.06	2.90	2.86	0.501 *
Fiber	19.6	22.06	20.50	22.60	2.197 *
CHO	38. 5	25.66	30.65	31.66	4.731 *
* (P≤0.05), NS: Non-Significant.					

Phytochemical assay

Results of this study presented the phytochemicals content of each (Fig, Olive and Moringa, FOM blend) as shown clearly in (Table, 2). It was founded each plant separately or in a combination state were contained alkaloids, tannins, flavonoids, phenols, glycosides, coumarins, trypsin and steroids. While, it was founded the saponin not founded in Olive leaf of both alcoholic and aqueous extract. Also, saponin content not founded in both alcoholic extract of both Olive and plants blend. These results come close with **(Shakeel *et al.*, 2015; Hamdia & Sundus, 2019)** .

Table (2): Phytochemical detection of each plant and the plant blend (FOM) extracts.

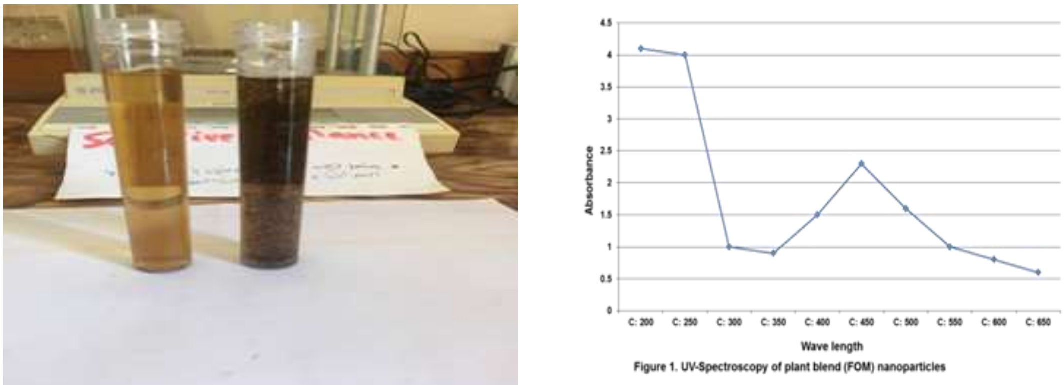
Phytochemicals	Moringa extract		Fig extract		Olive extract		Blend extract (FOM)		P-value
	Alcoholic	Aqueous	Alcoholic	Aqueous	Alcoholic	Aqueous	Alcoholic	Aqueous	
Alkaloids	+	+	+	+	+	+	+	+	NS
Tannin	+	+	+	+	+	+	+	+	NS
Flavones	+	+	+	+	+	+	++	++	0.044 *
Total phenol	+	+	+	+	+	+	+++	+++	0.006 **
Saponin	+	+	−	−	−	+	−	+	0.048 *
Glycosides	+	+	+	+	+	+	+	+	NS
Comorians	+	+	+	+	+	+	+	++	0.044 *
Ratingen	+	+	+	+	+	+	+	+	NS
Trypsin	+	+	+	+	+	+	+	−	NS
Steroids	+	+	+	+	+	+	+	+	NS
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.									

Synthesis of silver nanoparticles with blend plant

The synthesis of silver nanoparticles of the plant combination (Fig, Olive and Moringa) was confirmed using as shown in (Figure. 1), which shows the emergence of a band around 300 nm into the Nano synthesis of AgNPs (FOM).. Studies have proven that containing raw plant extracts for biosynthetic metabolites such as phenols, flavonoids, alkaloids, and terpenoids, which are mainly responsible for reducing the ionic mineral to a mass metal nanoparticle (**Chung *et al.*, 2016**). The results of the study showed the clarity of the synthesis of AgNPs by using a 5: 1 ratio of the extract of the plant blend (Fig, Olives and Moringa) by changing the color of the chemical reaction of the $AgNO_3$ solution from colorless to dark brownish-yellow at the peak of 350 nm in the UV spectrophotometer. This result comes close to (**Salari *et al.*, 2019**), due to different plant that were been used.

UV-Visible spectroscopy

UV-Visible absorption spectrometer. A UV–visible spectrophotometer was used in order to confirm the Nano synthesis of AgN(FOM)Ps. The identification of AgNPs was conducted by visual observation of the bright color of plant blend FOM extract with silver nitrate. The color changed from yellow to dark brown referred to the production of SNPs due to reduction of Ag^{+} ions as shown in (Figure 1, A and B). A double-beam UV-VIS spectrophotometer model (Shimadzu-1) was used to determine the optical absorbance spectrum of the FOM solution. Average size is determined for the silver nanoparticles created (NFOM) by performing UV-Vis spectroscopy by pumping (1 ml) of the sample into a test tube and analyze them at room temperature with a resolution of 1 nm and wavelength at 450 nm and after being decomposed by exposure to dynamic light using (Spectroscatter 201) Shimadzu type Ultraviolet Visible Spectrophotometer (UV-1100, Japan) as in (Figure 1b).



A B

Figure (1) (A &B): UV-spectroscopy Preparation of AgNPs nanoparticles

XRD-Diffraction

XRD –Ray diffraction was used to detect the crystallinity of green synthesized AgNPs prepared with medicinal plant extracts produced crystalline structure with average size 20 nm by exposing an X-ray crystal on the surface and interacting with the atoms. Atoms are arranged themselves at an appropriate distance on the crystal level and show the deviation pattern **(Jyoti *et al.*, 2016)**. The blend plant FOM sample showed strong reflections at 28.50, 40.10, 50.50, 58.20, 66.55 and 74.66 which referred to 112, 195, 221, 205 and 310 crystalline plane respectively as shown in (**Figure 2**). These results were similar to what he found of AgNPs prepared using Pedalium murex leaf (**Silva *et al.*, 2019**; **Jain *et al.*, 2021**).

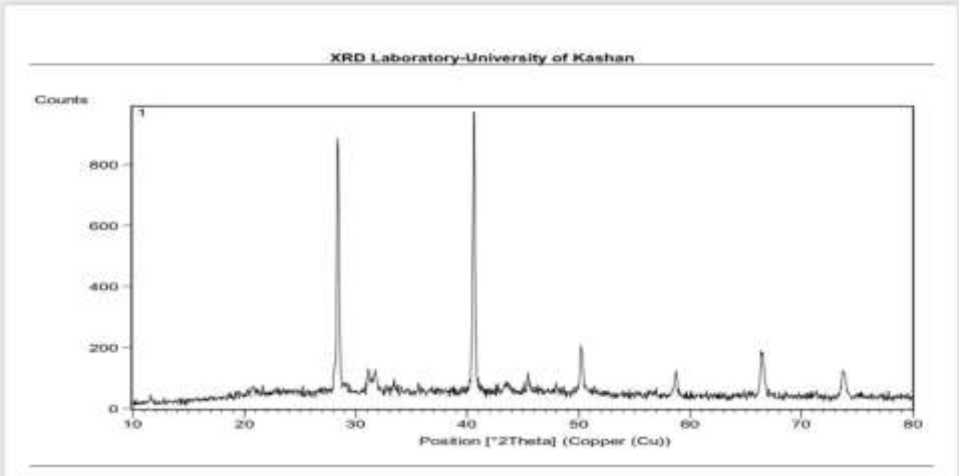


Figure (2): The blend FOM SNPs' X-ray diffraction pattern

Transmission electron microscopy (TEM) analysis

In order to determine the size of the biosynthesized NFOM particle, Zeta TEM analysis was performed to determine the average particle diameter in nanometer. NFO size distribution is shown in **(Figure 3)**. The size of NFOM particles ranged from 15 to18 nm with an average size of 16.5 nm. Furthermore, TEM **(Figure, 3)** demonstrated that most NFO were obviously spherical or polygonal in morphology. The results of an analysis selected area electron diffraction (SAED) pattern were similar to those found by (**Sangaonkar & Pawar, 2018**) previously obtained in some complex green AgNPs. The results of this study are almost similar to the previous studies, which used plant extracts as a reducing agent in the synthesis of AgNPs, and the nanoparticle size ranged from 44.18 to 71.57 nm **(Garibo *et al.*, 2020; Salari *et al.*, 2019; Jahan *et al.*, 2019**). Therefore, due to the very small nanoscale size, shape and high stability during nanosynthesis of AgNPs, these nanoparticles can be used in combination with drugs and targeting of cancer cells (**Ebtesam *et al.*, 2018**).

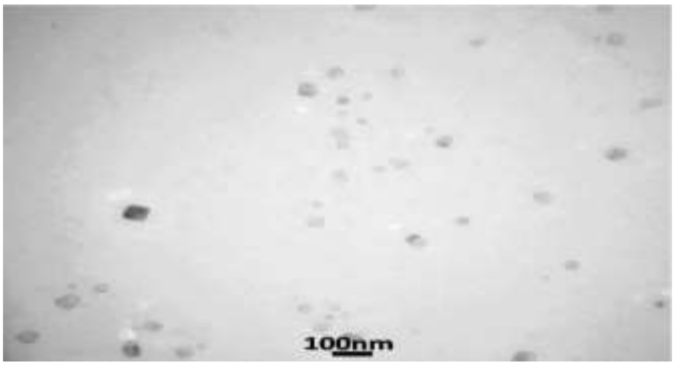


Figure (3): Transmission electron microscopy (TEM) of Nano (Fig, Olive and Moringa) blend particles.

Scanning Electron microscopy (SEM) analysis

Electron microscopy was used to determine the shape, size and morphology of nanoparticles synthesized from FOM with silver nitrate, as shown in **(Figure 4)**, which shows a regular SEM micrograph for synthesized nanoparticles of blend. The morphology of the monodispersed NPs is also shown without critical accumulation. The particle size average was 57.83 nm.

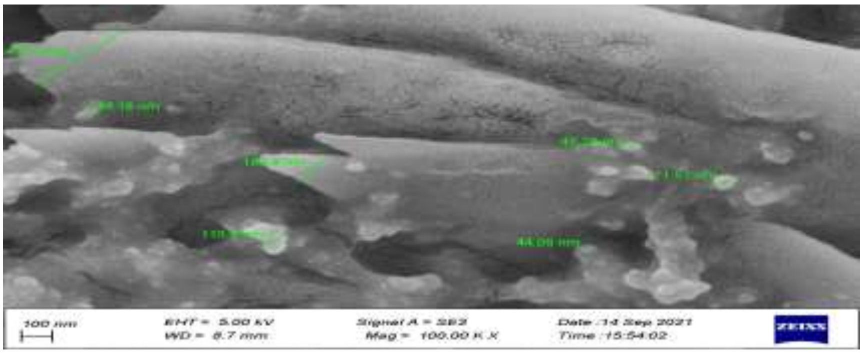
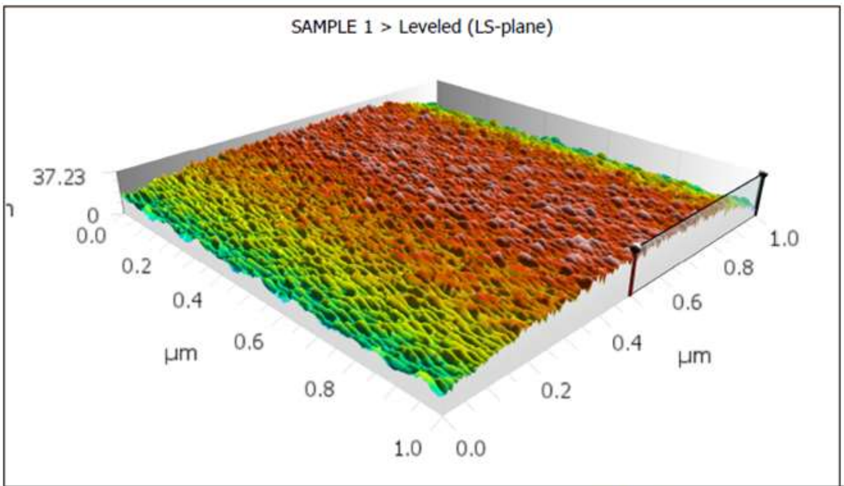


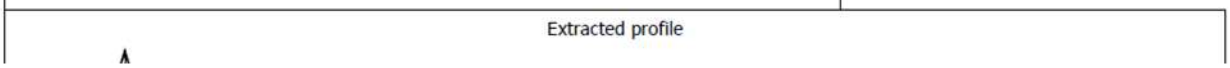
Figure 4: Scanning Electron microscopy SEM of Nano (Fig, Olive and Moringa) blend particles.

Atomic force microscopy (AFM) analysis

Atomic force microscopy is also used to analyze volume, surface morphology, structural and physical mechanical properties (**Silva *et al.*, 2019**). as shown in **(Figure 5).** The results of the AFM examination of nanoparticles synthesized using silver nitrate with extract of the plant mixture of leaves (Fig, Olive and Moringa) showed that the morphological surface of nanoparticles is rough and with pointed peaks and that the size distribution of the holes was Sq = 6.047 nm and Sz =37.23 nm as shown in **(Figure 5)**.



Axis:	X	
Length:	1.000	µm
Size:	256	points
Spacing:	3.922	nm
Axis:	**Y**	
Length:	1.000	µm
Size:	256	points
Spacing:	3.922	nm
Axis:	**Z**	
Length:	305.6	nm
Min:	-7435	nm
Max:	-7129	nm



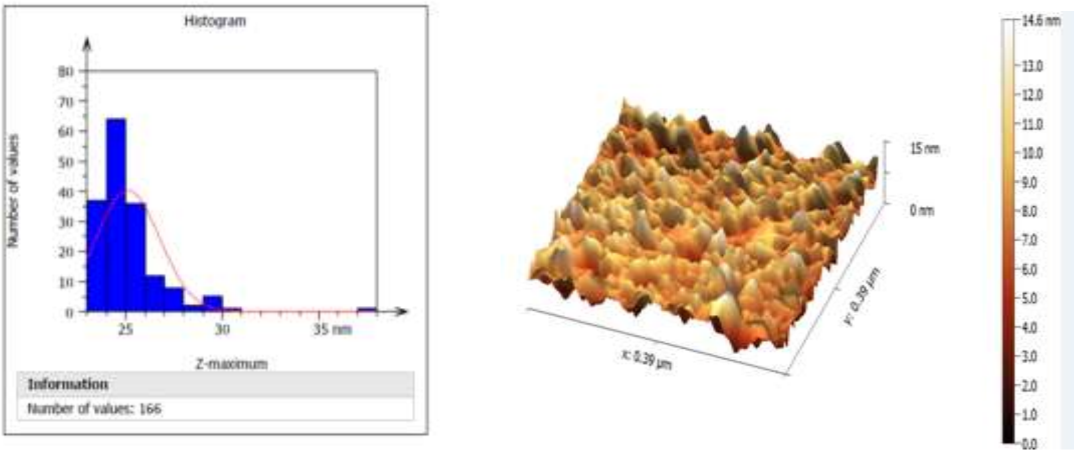
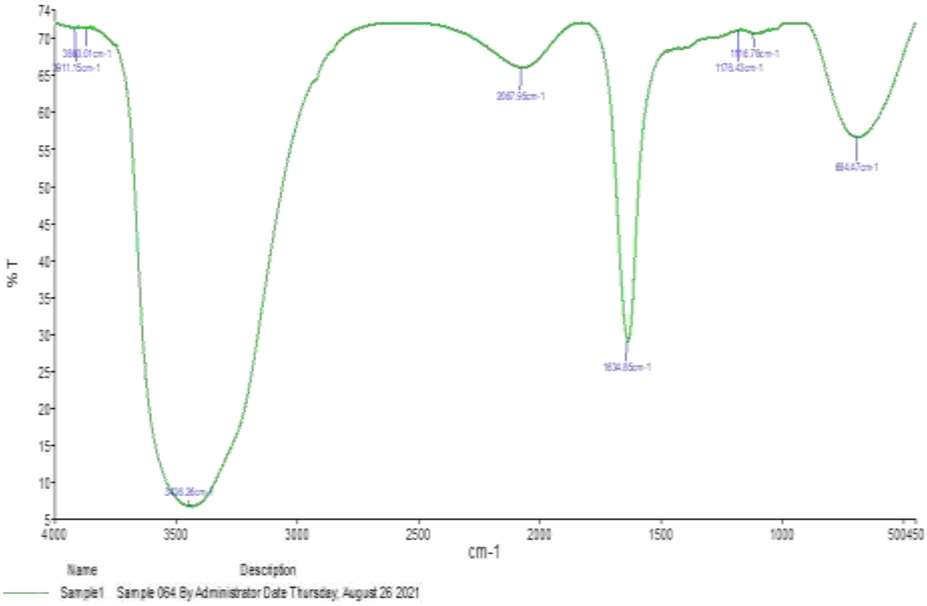


Figure (5): Atomic force microscopy (AFM) of Nano (Fig, Olive and Moringa) blend particles.

FTIR analysis

A Fourier transform infrared (FTIR) spectroscopy technique was used to evaluate chemical bonds in surface atoms and functional atoms can be on the surface of the nanoparticles were used to describe the physical properties of nanomaterial and their job (**Chung *et al.*, 2016**). Greenly synthesized AgNPs using leaf extract of plant combination of Fig, Olive and Moringa blend (FOM), shows major peaks by using FTIR which was proved the presence of some vital and effective organic compounds and form a strong layer as a coating on the nanoparticles, which appeared clearly in **(Figure, 6)**. The prepared nanoparticles were analyzed by FTIR to identify molecules that act as coatings and stabilizing agents, as well as to detect the reduction of silver ion. The results of this study also proved the presence of some bio-organic compounds which form a strong layer as a coating on the nanoparticles. This study showed major peaks at 3911.15, 3436.26 , 2067.95, 1116.78 and 684.47 cm $^{-1}$ which indicated the presence of different active groups such as carboxylic acid group (O-H), Alkynes group, ketone group (C=O), Alcohol and amide groups, and phenyl ring group respectively as shown in **(Figure 6)**. Result showed that amide and carboxylic functional groups may be sources for the reduction and capping resources in the green synthesis of AgNPs. The bond N-H stretching vibration of the NH2 group, and OH interference vibration of the attributed to water and blend leaf extract molecule. While the band in1634.85 cm $^{-1}$ corresponds to the band of amide stretching C=O and a peak at 2067.95cm $^{-1}$ can be assigned to the alkene group contained in the leaf of blend extract. The results of the study also showed that the band on 1178.43, 1116.78 and 684.47 cm $^{-1}$ was a strong peak with maximum intensity. The results are agreement with **(Mahdi *et al.*, 2015; Shakeel *et al.*, 2015**). Scientific studies have proven that various plant extracts possess the dual role of reducing and covering **(Shakeel *et al.*, 2015**). On the other hand, a study may have shown certain proteins and metabolites such as the existing terpenoids or flavonoids in *Prosopis juliflora* leaf extract, it may be responsible to degrade and stop the synthesis of AgNPs **(Altemimi *et al.*, 2017).**



Wave length (cm $^{-1}$)

Figure (6): Fourier transform infrared (FTIR) spectra analysis of synthesized silver nanoparticles with blend FOM extract.

Antimicrobial screening

Currently, it has been found that there is a high resistance of microorganisms to antibiotics. While there are many scientific studies on alternative solutions to curb their impact on human health (**Ibrahim, 2015**). Extensive studies have shown that phenolic compounds isolated from Olive leaves and many other plants have very good biological activities **(Khalil *et al.*, 2014)** including as special antimicrobials such as *Escherichia coli* and *Staphylococcus aureus* **(Mohammadi *et al.*, 2016)**. The results of using nanoparticles blend leaf extracts (Fig, Olive and Moringa, NFOM) extract had a high significant effect on bacterial inhibition through disk diffusion method as shown in (Table, 4). Silver nanoparticles showed higher microbial inhibition compared to the control sample or plant extract (FOM) alone. The reason is the very large surface area of the nanoparticles blend plant FOM, which provides better contact and penetrated inside the wall of microorganisms through its membrane **(Jahan *et al.*, 2019)**. This study showed that the most sensitive microbes are *Escherichia coli*, then *Staphylococcus aureus* against the extracts of the plant blend of (Figs, Olives and Moringa) synthesized Nano composed with silver nitrate which had significantly a high inhibition for almost all the concentrations used, and this is consistent with **(Dragana *et al.*, 2020).**

Table (4): Bacterial inhibition zone (mm) obtained by disc Diffusion method.

Bacterial isolates		**Inhibition zone (mm)**	**Inhibition zone (mm)**	**Inhibition zone (mm)**
		Control	**Nano-Botanical blend (Fig, Moringa & Olive**	**Botanical blend (Fig, Moringa & Olive) extraction**
***E-Colli* (-gram)**				
Dilutions	C: 100	Zero	23.2	8.1
	C: 75	Zero	20.0	3.0
	C: 50	Zero	12.5	2.2
LSD value		-	3.955 *	2.071 *
***Staphylococcus* (+gram)**				
Dilutions	C: 100	Zero	20.2	6.0
	C: 75	Zero	22.1	2.1
	C: 50	Zero	25.0	0.0
LSD value		-	3.882 *	2.156 *
* (P≤0.05).				

Effect of extraction agents on antioxidant activity

Used 2, 2-diphenyl-1-picryl-hydrazyl-hydrate to estimate the different active compounds simultaneously, it provides a simple and fast method for the evaluation of antioxidants by spectrophotometry (**Iqbal *et al.*, 2017; Dragana *et al.*, 2020).** According to many studies that have proven the effectiveness of phenolic compounds from various plant extracts, including Fig, Olive and Moringa leaves, they have big effects and working as antioxidants, especially oleuropein and hydroxytyrosol for those phenolic substances. There are previous studies that proved that plant extracts contain phenolic compounds such as tannins, flavonoids and phenolic acids, which showed a strong and effective activity and were considered as antimicrobials (**Almayouf *et al.*, 2020)**. The antioxidant activity of the blend leaf extracts (Fig, Olive and Moringa) was investigated using the DPPH scavenging method by measuring the total antioxidant capacity as in (Table, 5**)**. In this study, the effectiveness of (FOM) extracts and NFOM extract were compared with the synthetic antioxidant Butylated Hydroxy Toluene BHT and control. It was found that the alcoholic extract of Olive leaves has a high and significant effect (80.166%) in inhibiting the effectiveness of DPPH more than Fig and Moringa leaves, and it is very close to the standard BHT sample. The current study also demonstrated the efficacy of the plant combination with significant inhibition of the oxidative stress DPPH, as shown in (Table 5). This result comes close to (**Duong *et al.*, 2015; Ahmed *et al.*, 2016**). The results of this study were not in agreement with (**Hamad, 2015; Bethu *et al.*, 2018)**, who stated the percentage of Saudi Olive leaf inhibition was (52.0%) by extracting ethanol. The reason may be due to the differences between the varieties used. Extensive research has proven the effectiveness of natural antioxidants associated with oxidative stress as a preventative as well to treat many different diseases, because they contain phenolic compounds known for their antioxidant activity mainly, and they have oxidation and reduction properties. They are potential prevention agents treating many diseases associated with oxidative stress. The antioxidant activity of polyphenols is mainly due to oxidation and reduction. Also, the total phenols are hydrogen mangers, mono oxygen quenchers, and metallic chelates hemoglobin reduction Ferrell **(Mina *et al.*, 2020)** .

Table (5): Effect of different extraction parameters for DPPH scavenging capacity

Extract Concentration	DPPH radical scavenging activity %								LSD value
	Fig extract	Moringa extract	Olive extract	FOM	NFOM		Control	BHT	
C: 100	65.50	73.08	80.09	72.50	75.68		Zero	88.92	7.33 *
C: 200	66.86	74.56	82.50	73.00	78.55		Zero	88.92	6.92 *
C: 300	68.50	76.08	85.09	75.55	82.88		Zero	88.92	6.58 *
C: 400	70.45	78.28	85.50	78.92	85.65		Zero	88.92	6.04 *
LSD value	4.59 *	4.07 *	4.167 *	4.50 *	6.31 *		NS	NS	---
* (P≤0.05).									

CONCLUSION

High total phenolic content, total flavonoids, the main and bioactive compounds, which have proven effective as antioxidants and high inhibition of pathological bacteria such as *E. coli* and *Staphylococcus aureous* of this mixture of medicinal plants known since ancient times and mentioned in the Book of God (the Noble Qur'an) is of great importance to be exploited against many areas of life. Also it was presented the effect of alcoholic extracts from the blend leaves on exhibition of antioxidant and antimicrobial activity. Therefore, it has the potential to be used as a source of potential antioxidants and antimicrobials. And this study it gave valuable and important information against many different diseases, and therefore it is necessary to search and investigate more to exploit and apply these natural and inexpensive sources in many food products and natural remedies. We conclude from this study that these medicinal plants offer better things for treatment than industrial medicines to avoid their side effects and affect public health, but after studying and investigating more, and studying the toxicity of these plants in depth and intensive focus to exploit these available natural resources without any price.

COMPETING INTERESTS

The authors declared that there were no competing interests associated with the study.

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