https://doi.org/10.15218/hcchs.2023.04

A Novel Production of Multiple Phyto-Nanoparticles and Their Activity as Anti Head Lice and its Overlapping Bacteria

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Abstract

Background and objective: Nanotechnology is defined as a highly developed technology due to its wide range of applications in various fields of medical science, technology, and other research fields. Green synthesis methods are environmentally friendly, readily available, safe and non-toxic. The Objective of the present study is to producing nanoparticles from plant in addition to evaluation the effectiveness of the extracted nanoparticles as anti head lice and it is overlapping bacteria.

Methods: In this study, several nits were collected and more than 44 adult lice were screened for bacteria. Ethanol and Deionized water was used to obtain nanoparticles from Henna leaves, *Lawsonia inermis*. The characterization process was performed using UV-visible spectroscopy techniques, energy dispersive X-ray spectroscopy (EDX) and atomic factor microscopy (AFM). Pharmaceutical activity of the isolated nanoparticles was determined through using of agar diffusion test and *in vitro* study of the nit embryonic development.

Results: The results of the study include the identification of several nanoparticles from Henna leaf extract, including cadmium, iron, nickel, zinc, copper, cobalt, manganese, and silver, with different concentration density ranged from 10 nm to 50 nm, with a percentage of 80%. Obtained nanoparticles (especially 15µL in concentration) can penetrate the wall of the nit shell and reach the embryo followed by inhibition of it is growth also play a powerful role in eliminating and inhibiting the lice overlapping bacteria.

Conclusion: Development of a new drug product to treat and control the growth of head lice Phyto-multinanoparticles- is play a strong role in the eradication and inhibition of the nits and lice overlapping bacteria.

Keywords: Nanotechnology, Henna,Lawsoniainermis, Spectroscopy, Babylon.

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https://doi.org/10.15218/hcchs.2023.04

Introduction

Therapeutic plants are portion and divide of human society to combat illnesses, from the first light of civilization.[1] There exists a plenty of information, data and benefits of home grown drugs in our old writing of Ayurvedic(Conventional Indian Medication), Siddha, Unani and Chinese pharmaceutical.[2]

Nanotechnology is defined as a highly developed technology due to its wide range of applications in various fields of medical science, technology, and other research fields.[3] Green synthesis methods are environmentally friendly, readily available, safe and non-toxic. Nanoparticles are particles ranging in length from 1 to 100 nanometers in two or three dimension.[4]Nanotechnology alludes to the reshaping of matter at the nuclear or atomic level.[5]For the past few decades, there has been a significant investigate intrigued in particulate conveyance frameworks. So, particulate frameworks like nanoparticles have been utilized as physical approaches to alter and move forward the pharmacokinetic and pharmacodynamics properties of different sorts of drug molecules.[6] The application of phyto-nanoparticles extricates as decreasing operators will minimize the utilize of hurtful chemicals for nanoparticle union and a progressively common application of phytonanoparticle is their utilize as antimicrobial specialists in coatings, materials, wound dressings, and biomedical devices.[7]

Current study concluded the possibility of obtaining nanoparticles produced by the plant within the nutritional physiological activities, which can be obtained directly. This, in addition to the effectiveness of the extracted nanoparticles, plays a strong role in eliminating head lice and bacteria isolated from lice and inhibiting it. We call for the development of a new drug product to treat and control the growth of head lice parasites and prevent their spread.

Table (1): Equipment used throughout the study.

Equipment and Instruments	Company/MODEL	Country
Cold centrifuge	5702R	Korea
Electric shaker	ORBITAL2	UK
UV-Vis Spectrophotometer	UV-1700 (Shimadzu, Tokyo	Japan
X-Ray Diffract meter	Shimadzu XRD.6000	Japan
Atomic factor microscopy	AA2000	Japan

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Table (2): Tools and chemical substances used during study.

NO	Tools
2	Whatman filter paper No.1
3	Ethanol
4	Watch glass
5	Deionized water
6	NAOH solution
7	Indium Tin Oxide (ITO)
8	Fluorine-doped Tin Oxide (FTO)
9	Silicon slides
10	Acetone
11	Conductive polymer P3HT
12	Chlorobenzene
13	Metal rod

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Methods

Materials and Kits

The equipment, kits, and reagents that were used in the present study are shown in Table (1; 2) respectively.

Multiple Nanoparticle Preparation.

CollectionofHenna Leaves

Hennaleaves, *Lawsonia inermis* belong to Kingdome: Plantae, Order: Myrtales and family; Lythraceae.[2] were collected from local market in Erbil. Once it collected, it was washed and dried at room temperature for 5 days, then crushed to obtain powder.

PreparationofHenna LeavesExtract

In the current study, ethanol extraction method was used based on what mentioned by Hajji Nabih*et al*.[8] Dried leaves (30 grams) wereextractedwith120 ml of ethanol 95%. It was shacking for 2-3 dayusingashaker at 5-7^{O}C. Then, the extract was centrifuged (5000 rpm for 30 minutes) later filtered with Whatman filter paper No.1 finally centrifuged with cold centrifuge at 10,000 rpm. The supernatant separated and preserved at −4 °C. Powder product preparation were prepared through evaporation of final supernatant on watch glass at room temperature then diluted again by distal watertobe used in t experiments and characterization.

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Characterization of the Nanoparticles

UV-Visible spectrophotometer:

According to Quevedo*et al*. (2021),[9] the optical property of nanoparticle monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting of the sampleswith4mlof deionized water after regular period of time. UV-Vis spectral analysis was performed by using UV-Vis spectrophotometer UV-1700 (Shimadzu, Tokyo, Japan) that was operated in the scanningrange of 250-750 nm.

X-Ray Diffract meter (XRD).

X-Ray Diffract meter spectroscopy (XRD) analysis had been workingin College of Engineering, Babylon University. The elemental composition of all nanoparticles were investigated by using energydispersive X-rayspectroscopy(X-ray diffract meter Shimadzu XRD.6000 as 3K.NOPC) and it was confirmed by a formal letter of the University of Babylon. For XRD analysis the suspension of nanoparticles was dried into powder and about 1mg fine powder was used for the analysis according to Scimeca *et al*. (2018).[10] The XRD analysis Soft ware was sourced from Oxford instruments Analytical Ltd. All measurements were performed at an accelerated voltage of10 KV.

Atomic Factor Microscopy (AFM).

Depending on the protocol of Grobelny*et al*. (2009),[11] Atomic Force Microscopy (AFM) analysis accomplished for evaluation of the surface morphology, roughness and sizes rate of the isolated phyto-multinanoparticles which it had been working in Physics Department, Faculty of Science, Al-Nahrain University. The glass covered slides and silicon water were first cleaned in a 1 mol/L NaOH solution for 20 min. Subsequently, Indium Tin Oxide (ITO) coated glass, Fluorine-doped Tin Oxide (FTO) and silicon slides were cleaned using Deionized water, acetone, and ethanol, each for 20 min, and dried by clean air. The conductive polymer P3HT was dissolved in chlorobenzene at a concentration of 5 mg/mL with continuously stirring in a water bath at 80 °C for an hour. The PCPDTBT was dissolved in chloroform at a concentration of 5 mg/mL with stirring at 70 °C for an hour. Nanoparticles were first so nicated for 10 min to disperse them uniformly without aggregations, and then spin coated onto the glass, silicon, FTO, and ITO glass substrate at 4000 rpm for 30 s (in air). Samples of layers of the conductive polymers P3HT/PCPDTBT on ITO glasses substrates were prepared by spin coating at 3000 rpm for 60 s.

Anti Lice and Antibacterial Activity Test of the Prepared Multiple Phyto-Nanoparticles.

Anti Lice Activity Test

Nits (ova) of *P. human uscapitis* were collected from children (especially dark brown nits). Several hair attached nits were putt in a tube with Nano-particles which synthesized by alcoholic extract and the other placed on the slide and fixed by using the tape far from Henna extract (control). Daily embryonic developments were examination and followed up under microscope (40x) based on what mentioned by Sonnberg*et al*. (2010).[12]Approximately, six (6) days were required for fully embryonic development as reported by Al-Marjan*et al*. (2015).[13] Photos of the embryonic growth stages were taken in each day separately (using Camera Model,

https://doi.org/10.15218/hcchs.2023.04

HUAWEI NOVA 7i/ JNY-LX1, 2019) then compared with control.

Antibacterial Activity Test

Nano-particles which synthesized by alcoholic extract was tested for antimicrobial activity by using agar well diffusion method as described by Mollea *et al*. (2022).[14] Nutrient agar plates were used and swabbed with pathogenic organisms from fresh cultures using a sterile cotton swab. With the help of sterile metal rod drilling subsequently three adequately spaced wells (holes) of 6 mm diameter each were made per plate at the culture agar surface Pure cultures of microorganisms were sub cultured on nutrient agar then incubatedat37°Cfor24h.Attheend of the incubation period, the zones of inhibition were measured to the nearest millimeter. The inhibition zone is the area surrounding the hole with no growth of inoculated microorganisms.

Results and Discussion

Pharmaceutical Application of Phyto-Multinanoparticles.

In the present study, based on X-ray diffractive spectroscopy (XRD), UV-Vis spectroscopy and Atomic Force Microscopy (AFM) analysis, several phyto-nanoparticles were diagnosed from the Henna leaves, *Lawsonia inermis* including Cd, Fe, Ni, Zn, Cu, Co and Mn with different particle sized ranged between 27nm to 92nm (Figure 1) with a different concentration (Table 3). The reason for the low concentrations may be due to the extraction process and silver is not analyzed here for lack of the standard element. Analyzed curve of UV visible spectrophotometry in the mixture, where the results of the analysis of the prepared nano elements through examination by the method of measuring the visible spectrophotometry of the ultraviolet rays of the reaction mixture (Figure 2) that showed maximum absorption of visible ultraviolet rays in the range of 400-500 nm due to surface plasm on resonance of Ag, specifically within the 440-460 nm trend according to the published literature on silver analysis.[15] which indicates the presence of particles Nano-silvers with the highest density.

Table (3): Metal concentration before and after extraction from Henna leaf

Analysis condition	**Metals concentration in ppm**						
	Cd	Fe	Ni	Zn	Cu	Co	**Mn**
Before extraction	0.335	4.198	1.332	0.556	0.019	4.790	**18.590**
After extraction	0.206	0.445	0.267	0.399	0.014	1.090	**17.610**

https://doi.org/10.15218/hcchs.2023.04

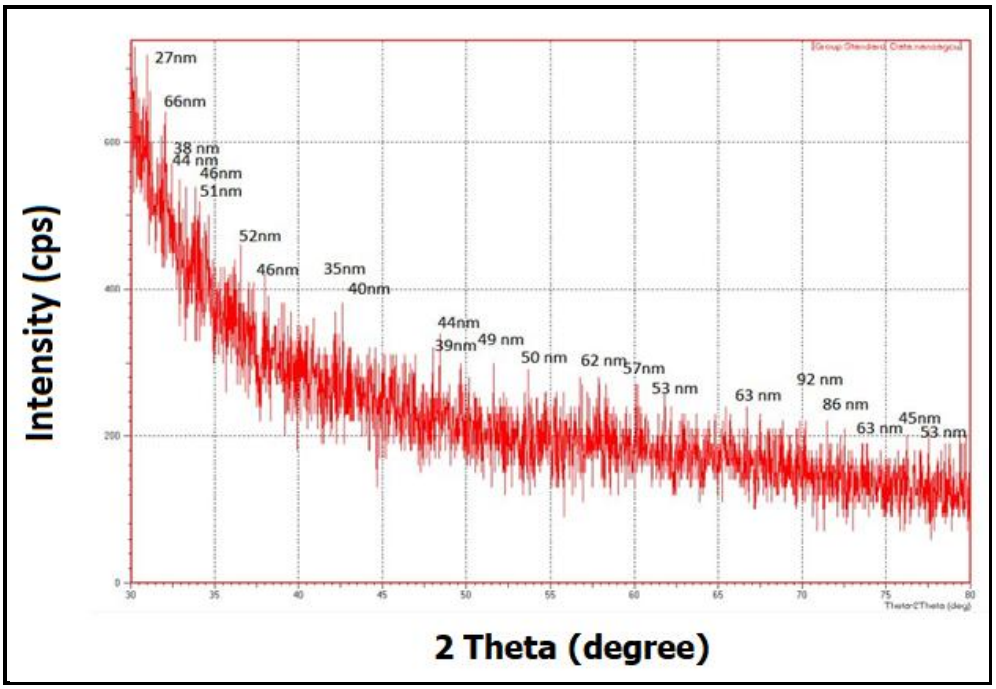


Figure (1): Sizes of prepared nanoparticles from Henna leaves, *Lawsonia inermis*, using X-ray diffractive spectroscopy (XRD).

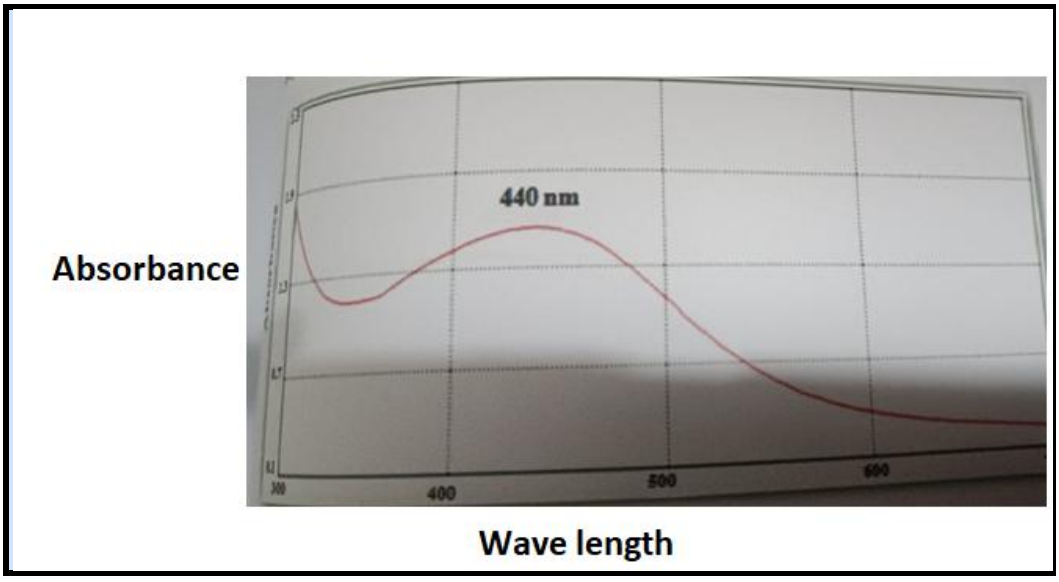


Figure (2): Ultra violet spectroscopy showed maximum absorption of visible ultraviolet rays in the range of 400-500 nm.

https://doi.org/10.15218/hcchs.2023.04

Atomic force microscopy (AFM) analysis accomplished for evaluation of the surface morphology and roughness of the isolated phyto-multinanoparticles, the result is displayed in Figure (3). The 3D AFM image indicates a fine stricter with maximum grain roughness size of 66.21 nm. A widely used parameter in AFM analyzing is the Root Mean Square (RMS). In this work, the RMS was 8.13 nm and this is very important point because roughness increase cell adhesion and it can help nanoparticles during host cell invasion.[16] The average size of nanoparticles and the beginning of around 10-50 nm in a rate 80%, but the other small percentage (20%) is approximately within the range 50-100 nm and the rest is larger as mentioned in Figure (4).

Figure (3): Atomic force microscopy (AFM) analysis of Henna extract, *Lawsonia inermis*

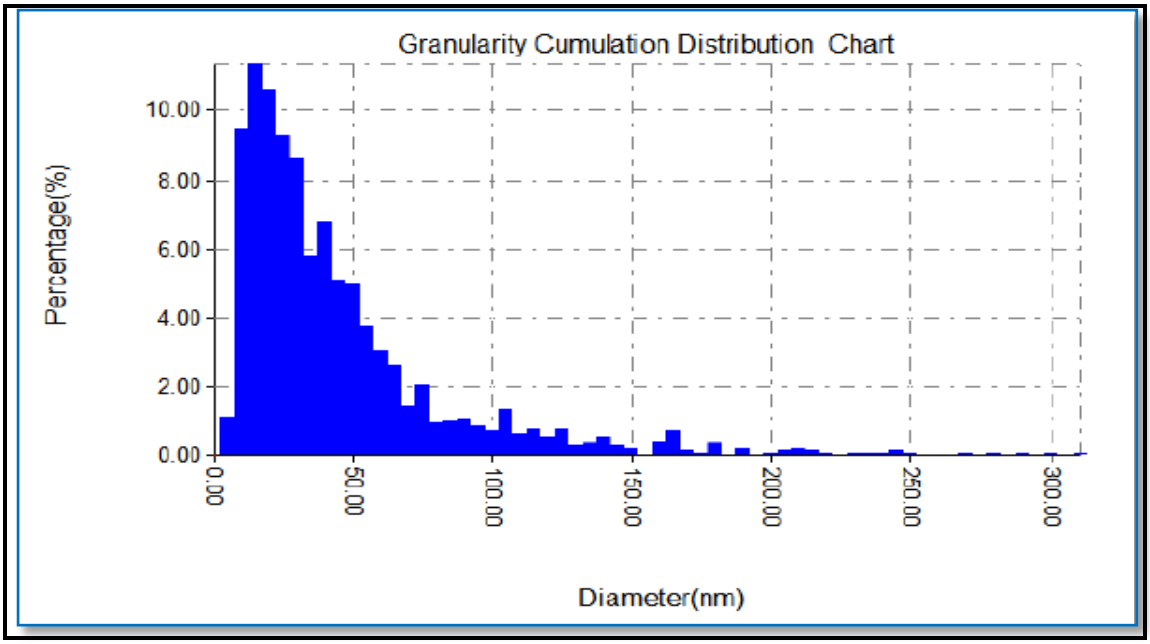


Figure (4): AFM micrograph of the Henna extract.

Sample of multinanoparticles were applied to nit and lice isolated bacteria in order to estimate its antiparasite and antibacterial activity. The following is an account and description of these application.

Pharmaceutical Application of Phyto-multinanoparticles Against Nit Development. Nit refers to the egg of the head lice which attached to the base of the hair in an affected person. It is covered by a thick shell wall and prevented entering of the any types of antiparasite. Nanoparticles can penetrate shell wall and reach to the embryo followed by retarding in its development and inhibit it. Sizes and surface roughness are among the most important physical properties of therapeutic nanoparticles which help nanoparticles during host cell invasion as mentioned by Xue*et al*. (2022).[16] In this study small size nanoparticles (<50nm) and

https://doi.org/10.15218/hcchs.2023.04

high range of root mean square (RMS=8.13NM) play an important role in the host cell adhesion and invasion process.

Steps of nit inhibition includes, at the first day, poor embryonic cell was appeared under the effect of multiple nanoparticles followed by degradation of embryonic cells and there are no any clear embryonic development and no nit hatching as mentioned in Table (4). Previously, several studies had been done related to nanoparticles and parasites, include those of Al-Marjan *et al*. (2019)[17] from Kurdistan region-Iraq, Sun *et al*. (2019)[18] from China and Kandeel *et al*. (2022)[19] from Saudi Arabia. No previous application of antilice activity of phyto-multinanoparticles in Iraq.

Pharmaceutical Application of Multinanoparticles Against Lice Isolated Bacteria.

In the current study, results of BLASTN and sequence similarity, confirm the isolation and identification of three genus of bacteria from the head lice including, *Acinetobacter gyllenbergii*, *Citrobacter freundii* and *Lysobacter oculi* (accession number, ON505830, ON126007, ON505828). Phyto-multinanoparticle activity was studied and estimated on all isolated bacteria. For these purposes, isolated bacteria were cultured on the nutrient agar with different volume (5µL, 10µL and 15µL) of isolated nanoparticles (agar diffusion test). After 24-48hrours of incubation, results appear that the nano-particles play a strong role in the eradication and inhibition of the isolated bacteria. Based on the diameter of the inhibition zone on culture media, no clear differences were recorded between using of 5µL and 10µL based on that the inhibition zone closely similar to each other (12.3mm and 15mm respectively), while there are clear differences between the using of 5µL and 15µL with the different inhibition zone (12.3mm and 20mm respectively) as mentioned in Table (5).In Iraq, during the last years, several studies had been done related to the estimation of the antimicrobial activity of the nanoparticles, including those of Al-Bazaz*et al.* (2018);[20] Alsultany and Mohaimeed (2021);[21] Al-Nema and Al-Ali (2022).[22] No previous study was recorded related to estimation of the phyto-multinanoparticles as antibacteria against *Acinetobacter gyllenbergii*, *Citrobacter freundii* and *Lysobacter oculi* in Iraq.

Several advantages of the new prepared Nanoparticles had been measured firstly, it is multi-element together in one process and from a plant source, secondly, obtaining nanoparticles with sizes less than 60 nanometers and by around 80%, in addition, it can be adopted for prevention to avoid injury because there are no harmful side effects, finally limited treatment time, reduce the cost and get rid of side effects. Results had been recorded in Center of Measurements and Quality Control, Management of Innovation and Industrial Samples in Baghdad.

https://doi.org/10.15218/hcchs.2023.04

Table (4): Nit Development under effect phyto-nanoparticles compared with control.

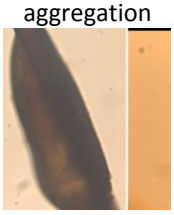
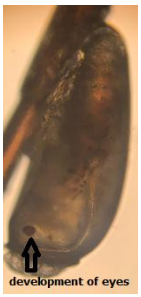
Duration / State	First Day	Second Day	Third Day	Fourth Day	Fifth Day
With multiple phytonanoparticles (test)	Poor cell development and cell Embryonic aggregation 	No development 	No development 	No development 	No hatching 
Without multiple phytonanoparticles (control)	Development of embryonic cells 	Aggregation of embryonic cell 	Development of eyes 	Development of legs  Development	Hatching process 

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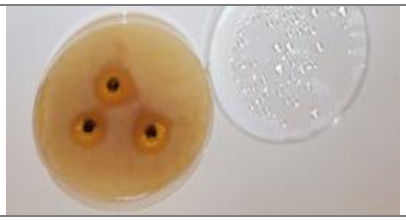
Table (5): Lice- isolated bacterial growth inhibition under effect of multiple phyto-nanoparticles.

Plant concentration	Volume	Incubation condition	Results	
	(microliter)	C°/hours	Inhibition zone in millimeter ($\bar{X}$-Diameter)	Culture properties
Extract 1 5mg	5	37C°/48	12.3	
Extract 2 5mg	10	37C°/48	15	
Extract 3 5mg	15	37C°/48	20	

Conclusion

Development of a new drug product to treat and control the growth of head lice Phyto-multinanoparticles - is play a strong role in the eradication and inhibition of the nits and lice overlapping bacteria.

Acknowledgements

I would like to extend my thanks to the Head of the Department of Medical Laboratory Technology, Erbil Technical Health and Medical College, Erbil Polytechnic University for their kind behaviors and cooperation.

Conflicting of interest

The authors have no competing interests to declare that relevant to the content of this article.

https://doi.org/10.15218/hcchs.2023.04

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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