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RESEARCH PAPER

Green synthesis and Larvicidal Activity of Citrus Aurantium Leaves Extract Nanoparticles Against Pediculus Humanus Capitis

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ABSTRACT

Resistance to insecticides and inadequate compliance with the application protocols of topical pediculicides often lead to treatment failure. Essential oils or plant extracts function as effective and safe alternatives due to their low mammalian toxicity and great biodegradability. Aim of the study: This study aimed to determine the pediculocidal and larvicidal efficacy of synthetic silver nanoparticles (AgNPs) derived from the aqueous leaf extract of *C. aurantium* against the head louse *Pediculus humanus capitis*. Materials and methods: Samples of head lice were randomly collected from several locations throughout the limits of Tikrit Governorate. A study was conducted on the hair of 150 children, both male and female, aged 2 to 12 years, to detect head lice infestations and evaluate the prevalence of the parasite in the area. Subsequently, the detection of the *COI* gene via polymerase chain reaction. This study utilized silver nanoparticles synthesized from *C.aurantium* leaf extract via a green synthesis process, with characterization conducted using UV spectroscopy, Fourier Transform Infrared Spectroscopy Analysis (FTIR), scanning electron microscopy(SEM) and x-ray diffraction (XRD). Result: The synthesis of AuNPs was confirmed via SPR as characterized by an absorption maximum at 435 nm. FTIR was used to estimate the biomolecular functional groups responsible for bioreduction of silver ions. XRD patterns of the AgNPs showed well-defined peaks at (211), (200), (220), (222) and (311) planes which provided an evidence to the face-centered cubic structure of silver crystallites. The particle sizes of the nanoparticles were in the range from from 23.06 to 38.48 nm and they have a nearly spherical shape, as indicated by SEM images. Nanoparticles derived from *C.aurantium* leaves had good outcomes at a concentration of 20 mg/L, resulting in greater fatality rates, followed by concentrations of 10 mg/L and subsequently 5 mg/L. Nanoparticles derived from *C.aurantium* leaves exhibited significant ovicidal action by postponing nymph emergence on the 6th and 14th days, ultimately inhibiting emerging entirely, akin to the standard. Conclusion: The green synthesis of nanoparticles considers an eco-environmental friend, safe, cheaper and easy to preparation. Synthesized AgNPs from *C. aurantium* extract have a high effect against adult insect and ovicidal activity by delaying the emergence of nymph.

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INTRODUCTION

Pediculus humanus capitis is an ectoparasite responsible for scalp infestation in humans, known as pediculosis. Infestations typically affect the pediatric population, particularly those aged 6 to 12 years, and involve feeding on human blood [1]. Head lice are small insects that live in human hair and survive by sucking blood through the scalp. Due to their small size, head lice are difficult to see with the naked eye at a glance. Their size varies, ranging from a pinhead to a sesame seed. The incubation period for lice eggs is approximately 8–9 days to reach adulthood, with each louse typically requiring 9–12 days to mature. Once fully grown, lice can live for up to four weeks before dying [2, 3]. Head lice take about 2 weeks to develop from egg to adult and start laying eggs again, so their rapid life cycle accelerates their reproduction on the scalp. Male head lice measure about 2 mm long, while female head lice measure approximately 3 mm. The total number of eggs laid during a female louse's lifetime is estimated to be around 140 [3]. Despite improvements in sanitation and the development of novel treatment methods, lice infestations persist in many communities, particularly in educational and densely populated settings [4]. Even with better hygiene practices and new medical therapies, lice infestations are widespread in many areas of the world, particularly in schools and other crowded locations [5]. Head lice treatment includes pharmacological, mechanical, domestic, and preventive approaches. Medical interventions often consist of shampoos and topical formulations including chemicals like permethrin, ivermectin, and malathion, particularly formulated to eradicate lice. Mechanical techniques, including routine combing, facilitate the elimination of lice. Moreover, personal hygiene and the regular laundering of linens and garments are essential preventive strategies. Treatment selection should depend on the severity of the infestation and the individual's condition [6]. Currently, chemical treatments represent the predominant method for lice management [7]. Concerns about parasite resistance to these substances and their adverse effects have prompted researchers to focus on traditional medicine and medicinal plants[8].

Citrus aurantium (*C. aurantium*) L. is a fruit-bearing citrus extensively cultivated in several places globally, especially in the Mediterranean and Southeast Asia [9]. It has attracted considerable attention in the scientific community because to its extensive variety of bioactive components, such as flavonoids, alkaloids, essential oils, and other phenolic compounds [10, 11]. These bioactive chemicals possess many pharmacological activities, including anti-inflammatory, antioxidant, antibacterial, and anti-obesity actions. *C. aurantium* has been widely utilized in traditional medicine and as a natural component in the culinary, cosmetic, and pharmaceutical sectors [12, 13]. Due to limited research has been conducted to evaluate the efficacy of *C. aurantium* leaves extraction as insecticides for managing ectoparasite infections. There fore this study aimed to evaluated the effect of *C. aurantium leaves* on *Pediculus humanus capitis.*

MATERIALS AND METHODS

Sample collection of head lice

Randomly collected samples of head lice from diverse areas on the borders of Tikrit Governorate. An examination was performed on the hair of 150 children, comprising both sexes, aged 2 to 12 years, to identify head lice infestations and assess the incidence of the parasite in the region. Active cases of lice infestation were examined utilizing a fine-tooth comb, relying on visual identification of living adult or nymphal stages, as well as viable nits, under natural sunlight [14-16]. An infestation was deemed positive if at least one active instance was documented [17]. The outcome was documented as negative for lice infestation if nymphs, adults, or viable nits were absent [17]. The samples were stored in 70% ethanol to maintain the integrity of the genetic DNA for optimal extraction quality.

DNA extraction

DNA was isolated and extracted from head lice samples using the extraction kit stored at room temperature and prepared by G-Spin/Korea for extracting DNA from blood cells, animal tissues, etc. Preparation was made to extract pure DNA extracted from animal tissues, kit number (17045) for DNA extraction. For *P. humanus capitis* as forward primer 5'-GGTACTGGCTGGACTRTTTATCC-3', and the degenerate reverse primer sequences were 5'-CTAAARACTTTYACTCCCGTTGG-3' [18]. The PCR reaction mixing solution (Taq RCR Pre MIX) is produced by Intron, Korea. The settings for PCR amplification were: initial denaturation at 95 degrees Celsius for Three minutes; forty cycles

of 95°C for one minute, fifty degrees Celsius for One minute, and 72 degrees Celsius for 1 minute; concluding with a final extension at 72°C for seven minutes. The PCR amplicons were examined via 1.5% agarose gel electrophoresis, stained with ethidium bromide, and subsequently visualized.

Preparation of C.s aurantium leaves extract

The extract prepared according to [19]. *C. aurantium* leaves were harvested and washed thoroughly with water and then washed with distilled water to remove any dust particles. The washed leaves were dried in oven at 50 °C for 4 hours. The dried leaves were crushed and sieved for less than 220 micrometer. The crushed leaves were extracted using deionized water by adding 100 ml of deionized water to 10gm of crushed leaves. This mixture was stirred for 1 hour at 60 ± 5 oC after that the mixture was then decanted and centrifuged in cooling centrifuge for 10 minutes at 12000 rpm then the aqueous extract was collected to use in the next step·

Silver nanoparticles synthesis

For the production of silver nanoparticles (AgNPs), mix 250 ml of deionized water with 2.00 g of silver nitrate (AgNO3) and heat them on a hot plate with magnetic stirring at 40°C. Then, 10 ml of the leaf extract was slowly added to the solution of silver ions. The color of the mix was changed to yellow after that. The hue of the solution made it easier to use UV-Visible spectroscopy to investigate absorbance and confirm the production of AgNPs [20].

Silver nanoparticles Characterization

UV- Visible spectroscopy

UV-visible spectroscopy initial characterizations of AgNps, including size estimate within the composition suspension were performed by UV-visible spectroscopy at center for advanced biotechnology and research (Shimadzu UV-visible 1900i, Shimadzu, Japan) [20].

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

The spectrum of the infrared spectrometers at the wave lengths (400-4000 cm) with a SHIMA DZU-Japan device, for all samples tested in liquid and at room temperature, according to [21] method.

Scanning electron microscope

A Carbon-coated copper grid was used for the microscopic analysis, and a minimal amount of sample were dropped onto this thick carbon layer of the grid. Then, the grid was observed under microscope to identify morphologies of prepared particles [22].

X-ray diffraction analysis

XRD was performed to identify and confirm the crystalline structure of the synthesized silver nanoparticles (AgNPs). The XRD patterns of the powdered samples were recorded using an X-ray diffractometer (Philips X'Pert-MPD, Netherlands) over a scanning range of 10°–80° (2θ). Monochromatic Cu Kα radiation with a wavelength of 1.5406 Å was employed for the

Fig. 1. Morphological of head lice.

diffraction measurements [23].

Pediculicidal activity

The nanoparticle solutions were diluted with double-distilled water to obtain target concentrations of 20, 10, and 5 mg/L. We used eighty adults and sixty nymphs, as well as controls in distilled water. Using a micrometer syringe, 0.02 ml of the test samples was carefully deposited on each louse in a glass plate. After that, the louse was put in a Petri dish with filter paper on the bottom and placed in a dark room. For one hour, all of the Petri plates were in a dark room with a temperature of 26 ± 0.5°C and a humidity of 70 ± 1% [24, 25]. After an hour, the plates were removed, 0.5 cc of distilled water was given, and then they were put in the chamber under the right circumstances. After 18 hours, the plates were examined under a dissecting microscope for any indications of lice movement, with immobility indicating mortality [26]. About the eggs, moving 15 at once to the test solutions and rinses. After

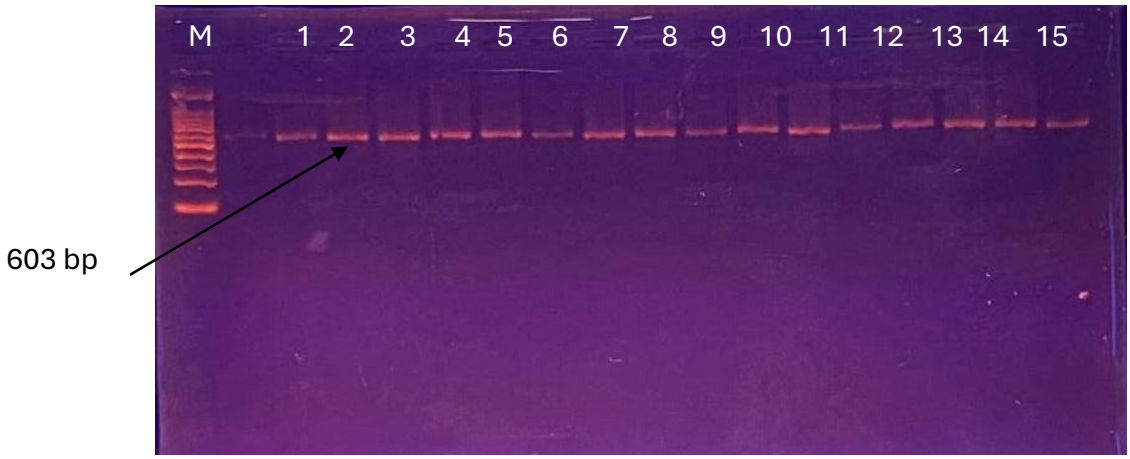

Fig. 2. The polymerase chain reaction (PCR) product has a 603 base pair band size. The product was obtained by electrophoresis on 1.5%, M: ladder represents DNA marker, and line = 1-16 represents head lice samples.

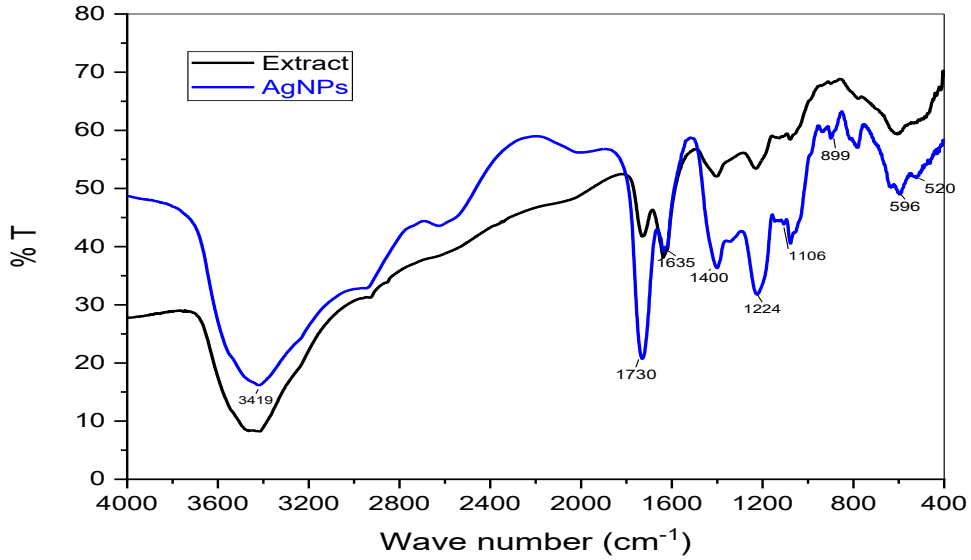

Fig. 3. FTIR of AuNPs and *C. aurantium extract.*

that, the eggs were swirled in several changes of filtered water and left to dry at room temperature. Then the eggs put in sterile glass vials (15 X 45 mm), sealed them, and kept them in the dark at 30 to 34 °C for two weeks. Every day. The ovicidal activity was measured by the number of eggs that hatched after 7 and 14 days.

RESULTS AND DISCUSSION

The present study showed prevalence of head lice from children aged 2 years to 12 years in Tikrit city were (54%) that diagnosed randomly.

Head lice were diagnosed using PCR technology to confirm the diagnosis of 60 lice samples, based on the primers *COI* in Fig. 2. The *COI* gene (603 bp) was diagnosed to study the genetic variation among them.

The FTIR (Fourier-transform infrared) spectra of extract obtained before as well as after the bio-reduction treatment (in Fig. 3) displayed several equivalent absorption bands at around 3429, 1730, 1630, 1400, 1225, 1106, 899, 596 and even at ha55 cm^{-1} wavelengths. The wide absorption band at ca. 3429 cm^{-1} was assigned to the NH stretching of amide group. An intense peak at 1730 cm^{-1} was attributed to C=O stretching of the carboxylic acid(–COOH) functional groups. The 1630 cm^{-1} lower band could be attributed to the amide I bands, coming mainly from carbonyl stretching of proteins.The band around 1400 cm^{-1} was assigned to the O–H and C–H bending vibrations, and that at about 1225 cm^{-1} appeared due to the C–O stretching of the carboxylic acid groups. The band at around 1021 cm^{-1} were assigned to the stretching vibrations of the C–N bond in amines. A dominant peak at 1106 cm^{-1} was attributed to C–O stretching of poly compound, polysaccharide, flavonoid and terpenoids, which are common in plant extracts that served as reducing agents during biosynthesis of AgNPs. The band at 899 cm^{-1} was attributed to aromatic C–H vibrations, and the low-frequency bands which were observed centrally around 596 and 520 cm-1 revealed Ag–O bonding, indicating the interaction of nanoparticles with oxygen containing functional groups. It is inferred from these FTIR data that certain bioactive substances in extract (such as flavonoids, phenolic compounds and alkaloids) are responsible for the reduction of silver ions to colloidal Ag (Ag^0). Additionally, these biomolecules serve as capping and stabilizing agents to avoid particle aggregation and maintain stabilization in water medium of AgNPs. This substantiates the two-sided role of constituents of extract used for the preparation and stabilization of silver nanoparticles[23].

The surface morphology of the features was characterized using SEM, as illustrated in Fig. 4. The characterization and examination of surface morphology indicated diameters varying from

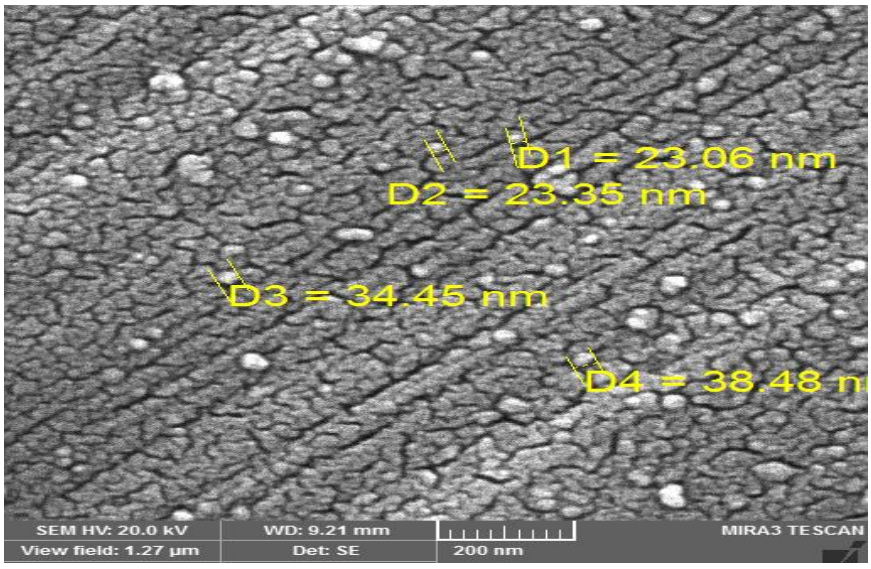

Fig. 4. SEM of silver nanoparticles.

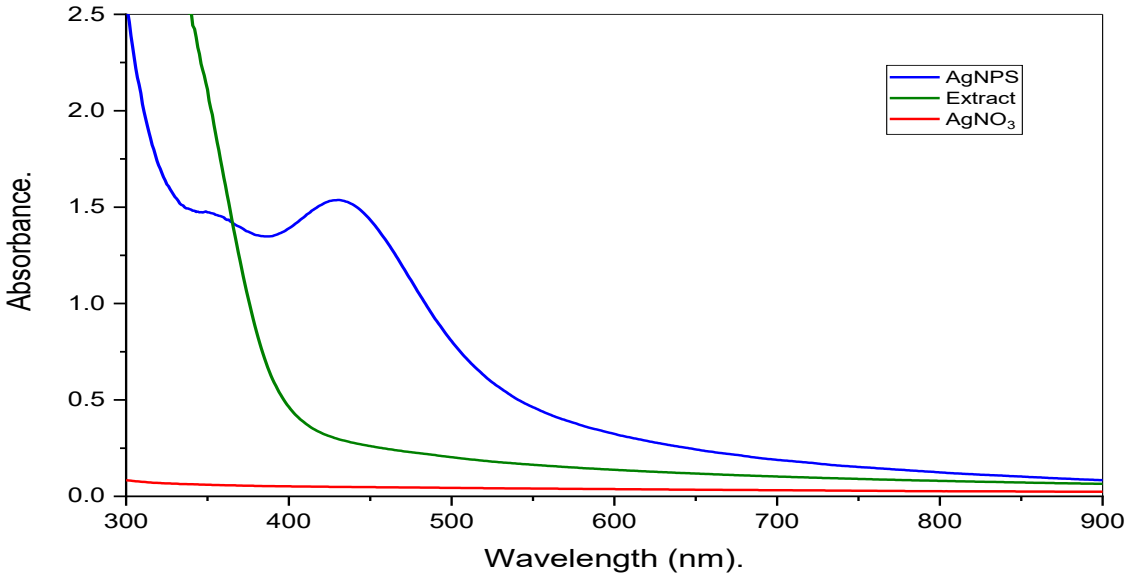

Fig. 5. UV visible spectrum of silver nanoparticles.

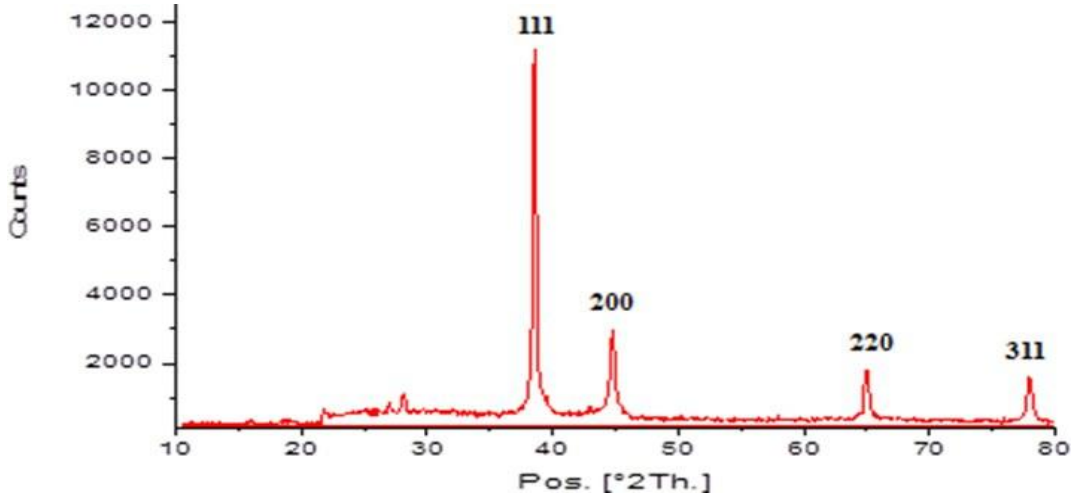

Fig. 6. XRD analysis of AgNPs.

Table 1. Effects of *C. aurantium* leaves nanoparticles against *Pedi culus humanus* capitisadults and nymphs.

Concentration	Average mortalitya (%)
Distilled water (0.5 ml)	10%
5	55%
10	80%
20	90%

Table 2. Effects of *C. aurantium* leaves nanoparticles against nymph of *Pediculus humanus capitis*nits.

Conc.	Emergencea (%)	
	Day 7	14
D. W (0.5 ml)	79.9	93.3
5	33.3	53.3
10	0	0
20	0	0

23.06 to 38.48 nm. The nanoparticles, being primarily nanoscale, demonstrate significant adsorption and reactivity owing to their diminutive size and elevated surface-to-volume ratio.

The silver nanoparticles produced from citrus peel extract were characterized via UV-visible spectrophotometry. For the formation of AgNPs, the UV–Visible absorption spectra is shown in Fig. 5. The plant extracts and $AgNO_3$ solution were employed for control. The presence of absorption peaks at λmax = 435 nm indicates formation of AgNPs. The efficient absorption of AgNPs in the ultraviolet spectrum indicates its prospective application in medicine.

XRD patterns of the AgNPs showed well-defined peaks at (211), (200), (220), (222) and (311) planes which provided an evidence to the face-centered cubic structure of silver crystallites. The average crystallite size of the synthesized AgNPs was estimated to be approximately 18 nm using the Scherrer equation[27], $D=0.94\ \lambda/\beta \cos\theta$, where D represents the crystallite size (nm), λ is the X-ray wavelength (Cu Kα = 1.5406 Å), β denotes the full width at half maximum (FWHM) of the diffraction peak, and ϑ corresponds to the Bragg angle associated with the (111) reflection. The sharp and well-defined diffraction peaks observed in the XRD pattern indicate high crystallinity and confirm the successful synthesis of pure AgNPs without detectable impurities.

C. aurantium leaves nanoparticles gave successfully results at concentration 20 mg/L that showed higher mortality followed by concentration 10, then 5. As shown in Table 2.

Nanoparticles derived from *C. aurantium* leaves exhibited significant ovicidal action by postponing nymph emergence on the 6th and 14th days, ultimately totally inhibiting emergence, akin to the standard.

The nanomaterial synthesized from *C. aurantium* leaves exhibited strong pediculicidal and ovicidal activity against head lice (*Pediculus humanus capitis*) and their eggs. The results showed a clear dose increasing the concentration and exposure time led to higher mortality rates. This suggests that the bioactive compounds present in the *C. aurantium* leaf extract possibly limonene, linalool, β-pinene, and other terpenoids contribute to the disruption of the lice's respiratory and nervous systems, leading to death. The small particle size of the nanomaterial may enhance its penetration through the insect's cuticle or egg chorion, increasing its effectiveness compared to the crude extract. Comparable insecticidal effects of Citrus species extracts and essential oils have been documented against several arthropods, including Anopheles stephensi, hence endorsing the viability of *C. aurantium*-derived nanomaterials as environmentally sustainable pest control agents [28]. The absorption of extracts into the alimentary tract of lice is negligible, as all extracts were applied to lice on filter paper, thus inhibiting substantial diffusion of active components into the cuticle when the substance is directly administered to the insect's integument [29]. Moreover, the lice were not placed in a limited setting, as the petri dish remained uncovered, hence limiting the possibility of volatile compounds being absorbed by the spiracles. Synthetic pediculocidal medications leave a residue on the scalp post-rinsing, which improves lice control but also fosters the development of resistance in lice [30]. Natural extracts from medicinal plants are recognized for their safety and efficacy, exhibiting limited resistance development due to their distinct mechanisms of action, hence strongly endorsing the safe application of *C. aurantium* as an efficient anti-lice drug [31, 32]. The complete eradication of lice would occur if the pediculocidal medicines also inhibit nymph emergence and

potentially eliminate the nymphs. Extracts of *C. aurantium* effectively delayed the emergence of nymphs and may facilitate the detachment of nits from hair prior to hatching[33]. Consequently, the findings of this research indicate a good prospect for utilizing *C.aurantium* extract as an efficacious option for the treatment of human head lice.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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