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Effectiveness of Six Selected Medicinal Plants Against Headlices, *Pediculus humanus capitis*: an *in Vitro* Study

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Abstract: In head lice infestation situations, inappropriate topical pediculocide application and insecticide resistance are typical causes of treatment failure. Essential oils and plant extracts have drawn interest as safer substitutes with easy biodegradability and minimal toxicity to mammals. This study aimed to evaluate the effectiveness of garlic and pomegranate extracts, as well as oils derived from garlic, clove, castor, black seed, and onion, in killing adult head lice. A filter paper diffusion method was conducted for determining the potential pediculocidal activity of the garlic and pomegranate extracts and oils of garlic, clove, castor, black seed, and onion. The results revealed that castor and black seed oils exhibited highly anti-lice activity, resulting in mortality 100% after 1 hour of treatment. Moderate efficacy was observed for G96, G50, and POM96 samples, leading to mortality rates ranging from 46.66% to 66.67%. However, GA, POM50, POMA, GO, CO, and ONO did not demonstrate anti-lice activity within the first hour. After 12 hours of treatment, all samples showed 100% mortality, indicating strong anti-lice effects. This study demonstrated highly significant pediculocidal effects of all tested samples, particularly castor and black seed oils, against head lice infestations.

Keywords: Anti-lice activity, filter paper bioassay, pediculocide, head louse, Pediculus humanus capitis, head lice, black seed oil, castor oil.

1.Introduction

Certain arthropods, like lice, mites, and so on, have the ability to multiply rapidly, which raises the risk of disease agents spreading within their population [1]. An infestation of *Pediculus humanus capitis*, commonly known as the human head louse, is a serious issue related to public health. Since the earliest Homo sapiens, head lice infestations caused by unsanitary conditions have had a detrimental impact on civilization for decades [2].

The head louse, *Pediculus humanus* capitis De Geer (Phthiraptera: Pediculidae), is a tiny ectoparasitic insect that has a blood diet from humans exclusively in the scalp and neck area. *Pediculosis capitis*, or head lice infestation, is a worldwide public health issue that predominantly afflicts children aged 5-11 years in developed as well as developing countries [3]. Head lice are most commonly transmitted by direct contact; head-to-head contact with an infested person is the main mode [4]. Head lice infestation can be treated with a wide range of products available across the globe. They range from real insecticides, which have in the past become less effective with time due to resistances developed, to silicones, which are often highly flammable or have drawbacks such as adhesiveness [1].

Originally from central Asia, garlic (*Allium sativum* L.), a bulbous plant belonging to the Lillaceae family [5]. Additionally, it is a therapy for viral and bacterial diseases in traditional medicine [6]. Furthermore, garlic has several pharmacological effects such as antiviral, antibacterial, antifungal, immunomodulatory, anti-inflammatory, anticancer, hepatoprotective, antiparasitic, and insecticidal effects. It has also been used to lower LDL cholesterol and treat illnesses including cholera and malaria [7, 8]. Many bioactive component groups, including organic sulfides, saponins, phenolic compounds, and polysaccharides, are responsible for the majority of garlic's therapeutic effects [6, 9].

Pomegranate peel (*Punica granatum* L.) is a good source of bioactive compounds like flavonoids, hydrolyzable tannins and phenolic acids. Pomegranate peel has been reported to be related to various biological benefits including skin and cardiovascular health improvement and anti-inflammatory,

anticancer, antiviral, antibacterial, and antioxidant activities [10-15]. Clove (*Syzygium aromaticum*) is the unopened flower buds that occur on Myrtaceae family clove plants [16, 17]. Apart from being used as a dentistry anesthetic, clove essential oil was also used to treat burns and wounds. Clove was discovered to have antiviral, antibacterial, antifungal, and anticarcinogenic effects in different studies [18, 19]. Certain compounds were also discovered in the essential oil of cloves such as eugenol, β-Caryophyllene, eugenyl acetate, ellagic acid, and α-Humulene [20, 21, 22].

Black seed (*Nigella sativa*), belongs to Ranunculaceae family and has a variety of medicinal uses. Many societies have been widely used to treat a wide range of illnesses related to the digestive system, respiratory system, cardiovascular system, immune system support, liver and kidney functions, and general well-being [23]. Three main categories of components are found in *Nigella sativa* (NS): phenylpropanoids, terpenes (mono- & sesquiterpenes), and several important fatty acids [24]. Thymoquinone, flavonoids, proteins, alkaloids (nigellicines and nigelledine), saponins (alpha-hederin), and many additional compounds are also present in NS [23].

Castor oil is a vegetable oil with multiple uses, it is derived from the seeds of the *Ricinus communis* plant, which belongs to the Euphorbiaceae family. A combination of esters of saturated and unsaturated fatty acids, including glycerol-linked palmitic, ricinoleic, oleic, stearic, linoleic, and linolenic acids, are present in castor oil. Numerous medical uses for castor oil, including antibacterial, antiulcer, wound healing, immune-booster, and degeneration of the bones have been studied [25].

Allium cepa, commonly referred to as onions, is a member of the Liliaceae family. In addition to its widespread usage as an antibacterial, it has been shown to have antidiabetic, anticancer, antiplatelet, antioxidant, antidepressant, and antihypertensive properties. It has also been shown to have neuroprotective, wound healing, antiobesity, antiparasitic, and antihypercholesterolemic properties. All parasites are efficiently eliminated by onions, which also inhibit the irreversible reductase of *Trypanosoma brucei* trypanothione [26]. Several phytochemical analyses of *A. cepa* have yielded various chemicals such as protocatechuic, ferulic, gallic acid, isorhamnetin, quercetin, and kaempferol [26]. The aim of the present study was to evaluate the effectiveness of garlic and pomegranate extracts, as well as oils derived from garlic, clove, castor, black seed, and onion, in killing adult head lice.

2.Methods

2.1Preparation of samples

Bulb of *Allia sativum* and peel of *Punica granatum* plant materials were obtained from Alhebshi Company in Al-Mukalla, Hadramout, then were authenticated and voucher specimen no. were given to the plant samples (G12023 and POM12023), respectively. The plants material was ground by electric blender and then extracted using ethanol 96%, ethanol 50% and distilled water to produce *Allia sativum* ethanol 96% extract (G96), *Allia sativum* ethanol 50% extract (G50), *Allia sativum* water extract (GA), *Punica granatum* ethanol 96% extract (POM96), *Punica granatum* ethanol 50% extract (POM50) and *Punica granatum* water extract (POMA), respectively. The plants material of each sample 100 g was extracted by maceration method with 1000 mL of each solvent separately in water bath at 45°C for 2 days. Extracts were filtered separately and solvents were evaporated accordingly [27]. *Allia sativum* oil (GO), *Syzygium aromaticum* oil (CO), *Ricinus communis* oil (CAO), *Nigella sativa* oil (BSO) and *Allium cepa* oil (ONO) were purchased from Green Mountain Company, Hadhramout, Yemen.

2.2 Collection of Head Lice

With the consent of their guardians, adults of *P. humanus capitis* were removed from a sample of kids aged 4 to 12 by raking a metal louse comb over certain areas of the scalp. The parasite adults were collected and gathered by meticulously extracting them from the metal comb teeth and placing them in a sterile plastic container. Head lice were collected and delivered to our laboratory. For at least a month, kids had not been treated with any pediculocide solution, only the louse comb [2].

2.3 Pediculocidal Activity:

The filter paper diffusion method was used to assess the pediculocidal activity of the selected samples [2]. The test organisms were divided into 12 groups of five lice each. The groups of lice were placed on the filter paper at the bottom of the petri dishes, which were left open. Each test sample was poured onto the lice in a volume of 0.5 mL (200 mg/mL), spreading as a thin layer covering an area of 4 cm^2. Group 1 received 0.5 mL of distilled water as a control. Groups 2 to 12 were treated with 0.5 mL of G96, G50, GA, POM96, POM50, POMA, GO, CO, CAO, BSO and ONO, respectively. The petri dishes were then placed in a dark chamber under conditions of 26 ± 0.5℃ temperature and 70 ± 1% humidity for 1 hour. After the specified time, each dish received 0.5 mL of distilled water and was returned to the chamber under the same circumstances. After 1, 6 and 12 hrs, the dishes were examined under a dissecting microscope to figure out if the lice still moving or not. The lack of movement was thought to be an indication of lice mortality. To verify the accuracy and trustworthiness of the data, all treatments were conducted in triplicate.

3. Results:

The yield percentage values of *Allia sativum* and *Punica granatum* plant extracts are shown in Table 1. Out of *Allia sativum* and *Punica granatum* extracts, G96 and POM96 showed the least values of yield percentages, while GA and POMA showed the highest values of yield percentages and this could be due to the high polarity nature of the water as a solvent for extracting polar compounds existing in the plants [28].

Table 1. Yield percentage of the plant extracts

Extracts	Colour	% yield
G96	Dark Yellow	13.08%
G50	Yellow	17.49%
GA	Light Yellow	22.13%
POM96	Dark Green	6.14%
POM50	Green	8.03%
POMA	Light Green	10.51%

The pediculocidal activities of G96, G50, GA, POM96, POM50, POMA, GO, CO, CAO, BSO and ONO in this study were evaluated after 1, 6 and 12 hrs and the findings are shown in Table 2. CAO and BSO samples achieved 100% average mortality after 1 hr compared to 0% average mortality for control group, while GA, POM50, POMA, GO, CO and ONO showed 0% average mortality after 1 hr. There was a moderate mortality activity after 1 hr for G96, G50 and POM96. After 6 hrs only G96, G50 and POM96 are achieved 100 % mortality. After 12 hrs, all samples revealed 100% average mortality except control sample. Control sample contained only distilled water and that is why used as control sample to compare with the tested samples.

Table 2. Effects of samples against *Pediculus humanus capitis* after 1, 6 and 12 hrs

Groups	Samples	Average mortality % after 1 hr	Average mortality % after 6 hr	Average mortality % after 12 hrs
G1	Control	0±0	0±0	0±0
G2	G96	53.33±11.54	100±0	100±0
G3	G50	46.66±11.54	100±0	100±0
G4	GA	0±0	73.33±11.54	100±0
G5	POM96	66.67±11.54	100±0	100±0
G6	POM50	0±0	73.33±11.54	100±0
G7	POMA	0±0	66.67±11.54	100±0
G8	GO	0±0	66.67±11.54	100±0
G9	CO	0±0	80±0	100±0
G10	CAO	100±0	100±0	100±0
G11	BSO	100±0	100±0	100±0
G12	ONO	0±0	80±0	100±0

4. Discussion

One of the most frequent medical insects that infest people globally is *Pediculus humanus capitis*. Children are the ones most affected, with five million new cases of head lice infestation reported every year. Neurotoxic synthetic pesticides such as malathion, permethrin, lindane and cabaryl are used globally as pediculicides for the treatment of head lice. Unluckily, there has been an increase in head lice resistance to neurotoxic pediculicides in a number of regions worldwide. Alternative pediculicides are of critical importance to cure head lice. Because of their great safety for children and minimal mammalian toxicity, alternative pediculicides derived from plants or herbs are now attracting the attention of researchers as new choices for treating head lice [29].

Because they are biodegradable and less hazardous to humans than chemical pediculicides, natural products are becoming more popular as alternatives for treating head lice. It has been determined that chemical pediculicides, such as malathion, are hazardous to human health and particularly poisonous to young children. Thus, efforts have been made to promote the creation of novel plant-based head lice prevention treatments. In previous studies, commercial pediculicide products based on plant extracts, such as Aesculo Gel® "L" (Cocos nucifera oil), Licatack shampoo® (grapefruit extracts), Nopucid Bio Citrus® (bergamot essential oil) and Wash Away Laus shampoo® (neem extracts), have shown high effectiveness against head lice [30].

Researchers found that grape fruit extract- and neem seed extract-based shampoos to be very effective for naturally infested human head lice in Egypt, regardless of the infestation stage. Headphones, helmets, and combs are suggested to be disinfected with ethyl alcohol or antihead lice products, while bed sheets, clothing, cloth toys, and towels are suggested to be washed in water over 50 °C [4].

In the current study, several plant derived samples were evaluated for their effectiveness in killing human head lice. The results showed that all samples tested achieved 100% mortality after 12 hr, and after 1 hr only CAO and BSO samples are achieved 100% mortality, while after 6 hrs only G96, G50 and POM96 samples are achieved 100% mortality. The majority of NS seeds' oil's active constituents, including thymoquinone, are responsible for most of the herb's characteristics. Numerous therapeutic potentials for black seed and its active ingredient thymoquinone have been suggested by animal models and cell culture studies. These include antibacterial, anti-cancer, antipyretic, analgesic, anti-fertility and contraceptive, anti-oxidant, anti-oxytocic, anti-inflammatory, and anti-tussive effects. The antibacterial properties of black seed include activities against viruses, parasites, fungi, schistosoma, and both gram-positive and gram-negative bacteria [23].

Several phytochemicals have been identified in garlic, pomegranate, clove, castor, black seed and onion as summarized in Table 3.

Table 3. Main active constituents of garlic, pomegranate, clove, castor, black seed and onion

Plant	Alkaloids	Saponins	Flavonoids	Terpenoids	Tannins	References
Garlic	+	+	+			[31]
Pomegranate	+		+		+	[32]
Clove	+	+	+	+	+	[33, 34]
Castor	+	+	+	+	+	[35]
Black seed	+	+		+		[36, 37]
Onion			+		+	[38]

+ indicated for presence

Important phytochemicals like flavonoids, alkaloids, sterols, tannins, and terpenes, such as quercetin, kaempferol, ellagic acid, gallic acid, ricinin, and rutin are found in *Ricinus communis* L., which is beneficial to health in many ways [39]. Furthermore, palmitic, ricinoleic, oleic, stearic, linolenic acid, and linoleic, connected to glycerol are among the esters of unsaturated and saturated fatty acids found in castor oil [25]. Moreover, undecylenic acid, γ-decalactone, ricinoleic acid, and sebacic acid [40]. For onion and garlic, within a span of just two days of treatment, some of the ingredients that are found in essential oils were reported to be 100% lethal to termites and fumigant in nature. The dominant sulfur compounds determined in the essential oils include diallyl trisulfide, diallyl disulfide, diallyl sulfide, β-caryophyllene, and eugenol, of which diallyl trisulfide is the most toxic [26].

Because crushed wild onion leaves contain sulfur volatiles from *Allium* spp., they repel *Diaphorina citri* adults. In the same way, *A. cepa* dry powder was crucial in reducing the amount of egg deposition. As an ovipositor, it repels *Phthorimaea operculella*. Alk(en)yl-cysteine sulfoxides, which are precursors to reactive thiosulfinates and disulfides—nonprotein sulfur amino acids derived from cysteine—are produced by *Allium porrum* (L.). Numerous insects are protected by them, including the *Acrolepiopsis assectella* leek moth. An essential defense against the plant's most formidable natural adversary is provided by the release of sulfur volatiles, which rise in proportion to the sulfur precursor propyl-cysteine sulfoxide sulfur compounds [26].

The composition of the extract was shown to be correlated with the high insecticidal activity of garlic bulb water extract against *Spodoptera litura*, as reported by Meriga et al. High content of phytochemicals and interaction between compounds of garlic were responsible for the insecticidal effect [41]. Different plant products, like *Cymbopogon nardus*, *Canaga odorata*, *Annona squamosa*, lemon, turmeric, and tea tree oil showed to have pediculicidal activity [30]. Overall, this study shows that the chosen plant materials and oils (G96, G50, GA, POM96, POM50, POMA, GO, CO, CAO, BSO and ONO), especially CAO and BSO, have promising pediculocidal properties. These materials and oils offer possible substitutes for safe and efficient head lice control.

5. Conclusion

All samples tested in this study exhibited pediculocidal effects, among all the selected samples, castor and black seed oil showed maximum effects evidenced by showing their pediculocidal effects in 1 hr. Hence, the results obtained from this research provide a promising scenario for using castor and black seed oil as a potential effective alternatives for treating human head lice. Further research is recommended to identify and isolate the active constituents responsible for the pediculocidal activity.

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Authors' Contributions

All authors contributed to the experimental and writing of this research. The manuscript has been read and approved by all the authors.

Conflicts of Interest

There is no conflict of interest regarding the submitted research article.

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فعالية ستة نباتات طبية مختارة ضد قمل الرأس (Pediculus humanus capitis): دراسة مخبرية

الملخص: في حالات الإصابة بقمل الرأس، يُعدّ الاستخدام غير المناسب لمبيدات القمل الموضعية ومقاومة المبيدات الحشرية من الأسباب الشائعة لفشل العلاج. وقد استقطبت الزيوت العطرية والمستخلصات النباتية الاهتمام كبدائل أكثر أمانًا تتميز بسهولة التحلل البيولوجي وانخفاض مستوى السمية للثدييات. استهدفت هذه الدراسة تقييم فعالية مستخلصات الثوم والرمان، بالإضافة إلى الزيوت المشتقة من الثوم والقرنفل والخروع والحبة السوداء والبصل، في قتل قمل الرأس. أُجريت طريقة انتشار ورق الترشيح لتحديد النشاط المحتمل لمبيدات القمل لمستخلصات الثوم والرمان وزيوت الثوم والقرنفل والخروع والحبة السوداء والبصل. كشفت النتائج أن زيتي الخروع والحبة السوداء أظهرا نشاطًا عاليًا في مكافحة القمل، مما أدى إلى نفوق بنسبة 100% بعد ساعة واحدة من العلاج. ولوحظت فعالية متوسطة لعينات G96 و G50 و POM96، مما أدى إلى معدلات نفوق تتراوح بين 46.66% و66.67%. مع ذلك، لم تُظهر زيوت GA و POM50 و POMA و GO و CO و ONO أي نشاط مضاد للقمل خلال الساعة الأولى. بعد 12 ساعة من العلاج، أظهرت جميع العينات معدل وفيات بنسبة 100٪، مما يشير إلى تأثيرات قوية مضادة للقمل. أظهرت هذه الدراسة تأثيرات قاتلة للقمل عالية الدلالة لجميع العينات المختبرة، وخاصةً زيت الخروع و زيت الحبة السوداء، ضد الإصابة بقمل الرأس.