Pharmacological effect of *Cassia auriculata* against *Pediculosis humanus capitis* infestations as a public health problem

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Abstract

Infestation with head lice is one of the serious health problems worldwide. The treatment failure in the last 5 years has attracted every researcher worldwide. Hence the current study was designed to evaluate in vitro pediculicidal efficacy of traditional medicinal plant Cassia auriculata flower extract. Various concentrations of flower extract were prepared and head lice were collected from infested individuals. Pediculicidal activity was tested by exposing lice continually in varying concentrations of the flower extracts to assess mortality rates, knockdown time and ovicidal effects. Phytochemical screening was conducted to screen the bioactive molecules responsible for the pediculicidal effects. Phytochemical screening reported the presence of various pharmacologically important bioactive compounds in the C. auriculata flower extract including flavonoids, saponins and tannins.

The study attributes the effective pediculicidal activity of C. auriculata flower extracts, which significantly suggest that it holds promise as an effective and alternative remedy for the pediculicides. Further research is recommended to understand the basic mechanism of action and isolation of effective and potential bioactive compounds from C. auriculata flower extracts to develop a safer and eco-friendly formulation against pediculosis to address a widespread public health concern.

Keywords: *Tanners cassia,* phytochemical, pediculicide, pediculosis, lice, contact toxicity

Introduction

Pediculosis is one of the serious health problems in many developing countries and commonly referred to as head lice infestation caused by *Pediculus humanus capitis*. Head louse infestation mostly affects the scalp and hair, where lice feed on human blood, causing itching and discomfort. Pediculosis is a global issue, because head lice is sucking the blood several times a day and their saliva is injected repeatedly which causes toxic effect in the infested individuals. It is particularly common among children aged 3 to 11 years.[12] The common way of transmission of head lice occurs via person to person directly and through indirect contact such as personal belongings, bed invested by nits or adult lice. Due to scratching, the pediculosis may cause various clinical implications including erythematous papules and secondary bacterial infection.[14]

Diagnosing pediculosis typically involves visual inspection for live lice and their eggs (nits) near the scalp. Number of pediculicides are available for the treatment and managing the head lice infestation such as malathion, permethrin and pyrethrins, however, due to resistance these products can be challenging and potential toxicity results in high treatment failures.[16] The primary caution to avoid the pediculosis is education, hygiene and regular inspection. Secondly the practices to avoiding direct and indirect contacts like not sharing the personal belongings and performing frequent inspection in schools and day care centers should be followed[23]. Education and awareness campaigns may help and may promote the informed approaches for prevention, early detection and treatment.[24]

Chemical treatment including permethrin and malathion has been used to eradicate the infestation and treatment of head lice. Due to resistance, the efficacy of these chemical products is diminishing in the lice treatment. Number of studies have reported that the resistance to permethrin and pyrethroids and complicating treatment procedures leads to develop potential pediculicides or alternative therapies.[15]

Without any chemical applications, the prevention strategies such as regular scalp care, mechanical removing and wet combing are often recommended for young children and those who are sensitive to chemical treatments.[32] Owing to the complexities of lice treatment, there are no effective and safe remedies and no effective solution for pediculosis. The reappearance of infestations, coupled with the discomfort and social stigma associated with lice, underscores the need for more effective and safer treatments. Over the centuries, medicinal plants have been applied in traditional medicine for their various pharmacological activities. Recently, the application of plant derived compounds in the treatment of pediculosis is increasing due to their safety concern, natural origin, minimal side effects and potential efficacy. [10]

Most of the plants produce number of bioactive compounds as secondary metabolites which exhibit good insecticidal, repellent, or growth-inhibiting effects on a variety of insects and pests, including lice. Being less toxic to human and the environment, natural products are often used as an effective alternative to synthetic chemicals and making them an effective formulation for managing head lice infestations.[33] Several studies reported that the application of essential oils

and plant extracts showed promising results in the treatment of pediculosis. For instance, tea tree oil extracted from *Melaleuca alternifolia* contains terpinen-4-ol, which has been shown to have significant pediculicidal activity.[9,26]

Similarly, neem oil (*Azadirachta indica*) is rich in azadirachtin, acts as a effective bioactive compound that disrupts the metabolism and reproduction of various insects. The essential oils extracted from other plants like eucalyptus (*Eucalyptus globulus*), lavender (*Lavandula angustifolia*) and peppermint (*Mentha piperita*) have also been reported for their effective treatment against head lice due to their insect-repellent properties.[26,30,34]

Even though, there is no conclusive solution for pediculosis, due to the variability in the concentration and composition of active ingredients in plant extracts. In addition, the formulation and application process also make it challenging to standardize the treatment procedures.[35] Development of resistance is one of the severe and significant concerns in the pest management strategy, hence, there is need for developing and highlighting effective and potential treatment.

The native and traditional plant *Cassia auriculata*, commonly known as Tanner's cassia, is now widely recognized for its various medicinal properties. In Ayurveda and other traditional medicine, the various parts of the plant, including leaves, flowers and bark, have been used in to treat a wide range of ailments such as diabetes, fever and skin diseases.[29] *C. auriculata* extract is rich in number of bioactive compounds, including flavonoids, tannins and saponins, which are believed to have insecticidal and antimicrobial properties. Several studies reported that extracts from *C. auriculata* significant prevents and kills lice suggesting that it may be a promising natural alternative to chemical pediculicides.[2]

Due to the natural origin and minimal side effects and lower chance of causing resistance compared to synthetic chemicals, *C. auriculata is* emerging as a safer option, especially for children and individuals with sensitive skin.[28] In these aspects, the present study aimed to evaluate the pediculosis effectiveness of *C. auriculata* against *Pediculosis capitis*.

Material and Methods

Collection and preparation of *Cassia auriculata* flower extract: Fresh flowers of *C. auriculata* are collected from the local markets of Al Zulfi, Riyadh province, Saudi Arabia. The purchased flowers are gently washed with normal tap water and then rinsed with deionized water to eliminate pollutants and dust before being shade dried. The dried flowers are crushed to a fine powder and stored in an airtight container.[31] The floral extract is prepared using with minor modifications. To begin, 10g of *C. auriculata* flower powder is added to100 mL of double distilled water in a clean conical flask. Heat at 70ºC using a magnetic stirrer. After cooling, filter the extract with Whatmann no. 1 filter paper and store it at -4ºC for future use.[27]

Preliminary phytochemical analysis of *Cassia auriculata* flower extract: Qualitative assessment of terpenoids, steroids, saponins, proteins, flavonoids, carbohydrate and phenolic compounds in the *C. auriculata* flower extract is determined through preliminary phytochemical analysis.[18]

Antioxidant activity: The DPPH technique is used to assess the free radical scavenging activity of *C. auriculata* flower extract, as previously described by Baliyan et al[6]. The *C.auriculata* flower extract (20, 40, 60, 80 and 100µg/ml) is added to a 0.1 M DPPH solution and incubated for 30 minutes at 37 °C. The proportion of radical scavenging activity (RSA) in the investigated samples is determined using spectroscopic analysis at 517 nm. Ascorbic acid is employed to provide a positive control. The subsequent formula is used to determine the percentage of RSA:

DPPH radical scavenging % = [(A0 – A1)/A0] × 100

where A0 is control absorbance and A1 is the sample absorbance.

Ferric reducing antioxidant power (FRAP) assay: The antioxidant activity of *C. auriculata* flower extract has been verified by FRAP assay using the method described by Benzie and Strain[7] with minor modifications. The crude extract (100 µl) is mixed with 250 µl of sodium phosphate buffer (200 mM, pH 6.6) and 250 µl of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$) and agitated for 20 minutes at 50 °C. After adding 250 µl of 10% TCA, the mixture is centrifuged at 650 rpm for ten minutes. Next, 20 µl of 0.1% $FeCl_3$ is transferred to the supernatant in a 96-well microplate. Phosphate buffer is used as the negative control and ascorbic acid as the positive control. Absorbance at 700 nm is used to forecast the drop-in activity.[7,13]

Collection of *Pediculus humanus capitis*: The samples used in this study are collected among school-aged children. Participants were selected from primary schools. Written informed consent was obtained from the parents or guardians of all children participating in the study. The selection criteria included children aged 6-12 years with no recent treatment for head lice infestation in the past four weeks.[3] Children were screened for head lice infestation by trained healthcare professionals using visual inspection under natural light. Hair is systematically combed using a fine-toothed lice comb. Presence of live lice or viable eggs (nits) is the criterion for infestation.

Infested children are identified and lice are collected using a fine-toothed lice comb. Collection is performed by systematically combing the entire scalp, starting from the nape of the neck and moving towards the front.[1] Collected lice are transferred into labelled, airtight plastic containers for transportation to the laboratory. Lice are counted and

identified under a stereomicroscope to confirm species as *Pediculus humanus capitis*. Lice are classified into different life stages (adult, nymph and egg).

Topical application assay: Adult lice are selected randomly from the maintenance culture (20/plate). Various concentrations (25,50,75,100 µL) of *C. auriculata* flower extract are applied directly onto the dorsal surface of each louse using a micro applicator. Treated lice are placed in Petri dishes lined with filter paper and maintained under the same conditions as the maintenance culture. Mortality is recorded at 1, 2, 4-, 8-, 12- and 24-hours post-application. Lice are considered dead if they show no movement when prodded with a fine brush.[17]

Contact toxicity bioassay: A filter paper impregnation method is used for the contact toxicity assay. Filter papers (Whatmann No. 1) are cut into circular discs (5 cm diameter) and impregnated with various concentrations (25,50,75,100 µg/ml) of *C. auriculata* flower extract. The impregnated papers are allowed to air dry completely to evaporate the sample. 20 adult lice are placed on each treated filter paper within Petri dishes (9 cm diameter). Each treatment is replicated three times to ensure reliability and reproducibility of results. Petri dishes are sealed with parafilm to prevent the escape of lice and maintained at the same conditions used for their maintenance. Mortality is recorded at intervals of 1, 2, 4-, 8-, 12- and 24-hours post-treatment. Here in this, the organism is considered as dead if they showed no movement.[5]

Acute dermal irritation/corrosion assay: The irritation potential of *C. auriculata* flower extract was evaluated with acute dermal irritation assay. The assay performed is based on the established guidelines for dermal testing and was adopted to the present study requirements to determine the effectiveness of flower extract on head lice. The methodology was strictly followed the protocols were outlined by Organisation for Economic Co-operation and Development (OECD) with slight modifications suitable for ectoparasites.[20] Lice were acclimatized in a controlled environment at 28°C ± 2°C with 70-80% relative humidity for 24 hours before the assay. Human hair samples were used as substrate for the study, initially the hair samples were thoroughly cleaned by washing and dried. Various concentrations of *C. auriculata* flower extract were prepared ranging from 25, 50 and 100µg/mL and were applied to the substrate in a thin and uniform layer using sterile applicators. Similarly, the test samples were also applied to separate substrates.[21] Typically 20 individuals of adult head lice were placed on the treated substrates to ensure consistent exposure. Lice were exposed to the substances for 4 hours, as per OECD guidelines (OECD Test Guideline 404, 2015). After 4 hours of exposures, the lice were examined for any sign of irritation or behavioral changes.[36] All the lice were removed from the substrate and placed in a clean environment and monitored for additional 72 hours to inspect any irritation or adverse effects. Based on the standardized criteria adapted from OECD guidelines, the degree of dermal irritation was scored specifically focusing on erythema and edema.[25]

Results and Discussion

The crude extract crude flower extract from *C. auriculata* shows significant efficacy against head lice, hence it gained great attention due to their growing concern over the development of resistance to synthetic chemicals. Over the centuries, these plants were used in traditional medicine to cure various ailments. Recent studies also reported that it shows significant natural insecticides, especially against ectoparasites including head lice. In the present study, the efficacy of crude flower extract of *C. auriculata* against head lice was evaluated by calculating the percentage of mortality. Experimental lice treated with various concentrations lay considerable changes ($p < 0.001$) compared to control lice.[4]

Preliminary phytochemical screening: The presence of various phytochemicals was assessed through simple biochemical tests. The result revealed the presence of terpenoids, steroids, saponins, proteins, flavonoids, carbohydrates and phenols (Table 1).

Antioxidant property of crude flower extract of *C. auriculata*: Haemostatic metabolism occurs when free radicals are balanced via the antioxidant system. However, excessive free radical production produces oxidative damage, which can lead to a variety of chronic disorders including cancer, inflammation and diabetes.

Table 1
Phytochemical screening of *C. auriculata* crude flower extract

S.N.	Phytochemical	Presence/Absence
1.	Protein	Present
2.	Saponins	Present
3.	Flavonoids	Present
4.	Phenol	Present
5.	Terpenoids	Present
6.	Steroids	Absent
7.	Glycosides	Present
8.	Carbohydrate	Present

As a result, researchers are increasingly concentrating on natural antioxidants. In this study, the DPPH free radical was utilised to investigate the antioxidant capacity of a crude flower extract of *C. auriculata*.

The results showed that the deep violet colour of the DPPH solution gradually changed to pale yellow after being exposed to crude extract and ascorbic acid, indicating that the components have antioxidant properties.[19] The spectrophotometric measurements revealed that crude extract had much higher free radical scavenging performance than the control. The absorbance value decreased considerably with increasing crude extract content (Figures 1 and 2). Simultaneously, higher RSA was seen as the incubation duration rose.

According to researchers, the extract from *U. fasciata* has increased radical scavenging capacity as the concentration increases. Researchers found that at 400 µg/ml, *Ulva lactuca* has 75.02% antioxidant activity. As previously reported, crude extract was capable of reducing the power of free radicals. Indeed, crude extract has more antioxidant potential. The transfer of electron density to DPPH molecules may be responsible for the antioxidant action of crude extract.[19]

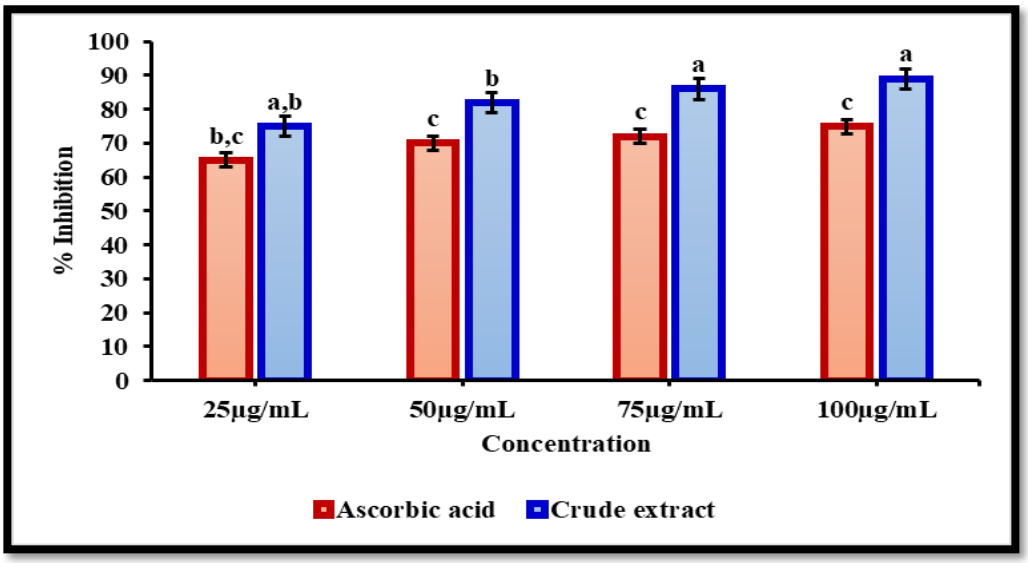

Figure 1: DPPH radical scavenging activity of crude *C. auriculata* flower extract. Experiment was performed in triplicate and the results were expressed as mean ± SD. Bars labelled with different letters represent statistically significant results ($p \leq 0.05$).

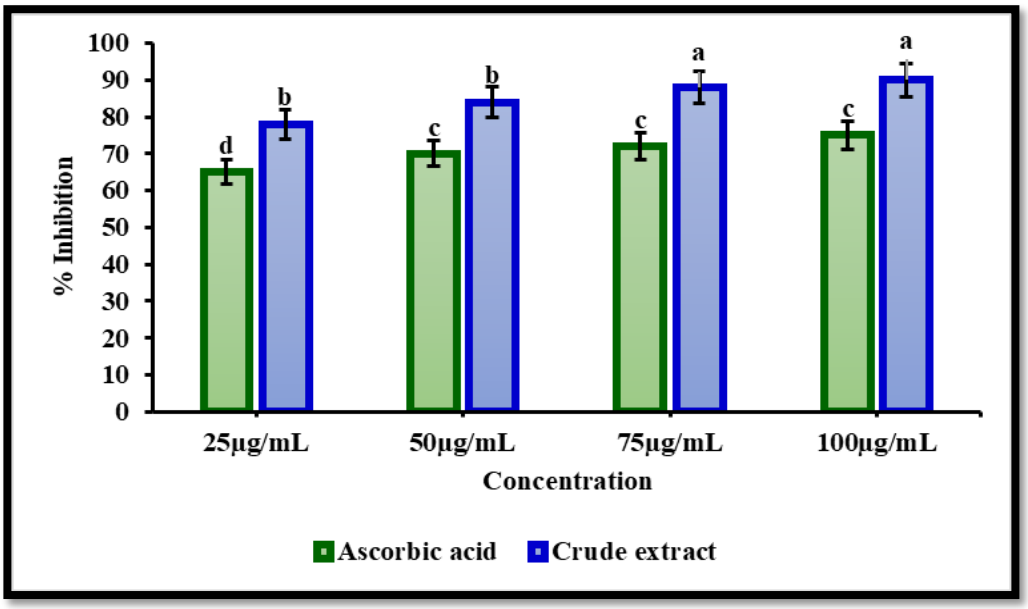

Figure 2: Free reducing antioxidant power (FRAP) assay of crude *C. auriculata* flower extract. Experiment was performed in triplicate and the results were expressed as mean ± SD. Bars labelled with different letters represent statistically significant results ($p \leq 0.05$).

Table 2
Pediculicidal activity of different concentrations of *C. auriculata* crude flower extract on *Pediculus humanus capitis*

Treatments	Number of dead head lice in different time observations (hours)						Mean ± SD
	1	2	4	8	12	24	
Control	0	0	0	1	1	2	0.66 ± 0.81
25 µL	2	2	4	7	9	10	5.6 ± 3.50
50 µL	3	4	8	10	11	12	8 ± 3.7
75 µL	3	6	6	10	14	14	8.83 ± 4.57
100 µL	4	9	11	12	16	16	11.3 ± 4.5

Topical application assay: The topical application assay of *C. auriculata* crude flower extract and its effect on head lice mortality demonstrate a significant increase in mortality rates among lice treated with the highest concentration of the crude extract when compared to a non-treated control group, which further highlights its efficacy as a natural alternative for controlling infestations. Crude flower extract of *C. auriculata* treated head lice showing alterations in the external physiology while in the control, no changes were observed.[11]

In addition, shrinkage of the head lice and abnormality in their legs were also observed in the treated group (Table 2). The extract's high efficacy is attributed to the presence of bioactive compounds such as flavonoids, alkaloids and tannins, which are known to exhibit insecticidal properties. These compounds are believed to interfere with the nervous system of the lice, leading to paralysis and eventually death.

The higher concentration significantly increases the lice mortality compared to control group. In control group no death was noticed which clearly depicted that the high dose of flower extract of *C. auriculata* may be used as promising agent to control the population of head lice. Moreover, the present study suggested that the *C. auriculata* crude extract may serve as an eco-friendly and safer alternative to the synthetic pediculicides, which are often used and cause environmental hazards and their associated side effects including skin irritation. Plant based treatment and traditional remedies are safe and less likely to cause resistant in lice populations, whereas the chemical products are associated with severe side effects and resistance.

Head lice infestation is one of the sever health concern in school-aged children, hence the natural treatment like using medicinal plant-based extract like *C. auriculata* extract offers a safer solution for managing the head lice infestation without any risk of skin irritation when exposed to young children. However, further investigation to develop the optimal concentration and formulation of the flower extract is needed to ensure the maximum efficacy and to minimize the potential adverse effects. This underlines the substantial potential of *C. auriculata* crude flower extract in dealing with head lice infestations. The higher dose of extract achieved a substantially higher mortality compared to the control group, which suggests that the *C. auriculata* crude flower extract shows the significant pediculicides effect in dose dependent manner, hence the natural remedy could be an effective alternative method to conventional pediculicides.

Several studies also reported that the other medicinal plants including *Azadirachta indica* (neem) and *Ocimum sanctum* (holy basil), show effective pesticides properties, though compared to other plant-based extract *C. auriculata* crude flower extract shows more efficacy in lower concentrations than other plant-based extracts. These findings support the previous report on botanical insecticides which highlight the potential effect of plant-based products as alternative and used in lice control owing to their less toxicity. Similar findings were reported in other plant extracts, including *Azadirachta indica* (neem) and *Mentha piperita* (peppermint), which also exhibited convincing pediculicidal effect due to their rich phytochemical content.[8,34]

Hence, the natural treatments are gaining great attention as alternatives to chemical products like permethrin, one of the commonly resistant products used in lice infestation. Overall, the present study demonstrated that the *C. auriculata* and similar plant-based extracts offer promising natural solutions in the treatment of lice infestation with less side effects. Therefore, the development of plant-based insecticides with formulation of *C. auriculata* extract could play a pivotal role in combating head lice and other parasitic infestations.

Contact toxicity bioassay: The eradication and prevention of head lice infestations are persistent issues worldwide, leading to the exploration of various plant-based solutions. *C. auriculata*, commonly known as Tanner's Cassia is one of the promising plants which was traditionally used for various pharmacological purposes. The effectiveness of *C. auriculata* flower extract at different concentrations 25, 50, 75 and 100 µg/ml on head lice exposure was presented in figure 3. After exposure to higher dose (75 and 100 µg/µl) of flower extract, it showed a high percentage of mortality compared to the non-treated control group.

Furthermore, we have noticed the behavioural changes like no contact between lice and isolation of the lice in the corner of the Petri dish. It strongly depicted that the higher dose of flower extract significantly prevents the contact and isolates the individuals. This highlights the efficacy of the *C. auriculata* as potential in the treatment of head lice infestation and its role in preventing lice contact and promoting the separation of affected individuals.

Head lice exposed to the crude flower extract of *C. auriculata* shows noticeable changes in external physiology. The lice treated with extract showed a clear deviation from normal behavioural changes and physical appearance compared to control group. In the current experiment, the treated group lice with exposure of flower extract became sluggish, with noticeable morphological changes such as shrinking of the exoskeleton and discoloration. This morphological change could be attributed to the phytochemicals present in the flower extract, which may have effectively penetrated the outer cuticle of the lice and leads to considerable physiological stress and eventual death.

In contrast, the control group lice did not exhibit any significant external changes. Also the control group maintained their normal activities and no signs of physiological distress were seen. The lack of external changes and behavioural changes in the control group underline the specificity of the response to flower extract of *C. auriculata* which suggested that the bioactive compounds present in it effectively target the lice specifically and potentially by disrupting their nervous system and respiratory systems.

The lack of contact between the lice is one of the interesting findings in the experiment. In typical lice populations, individuals tend to congregate and engage in frequent interactions which is vital factor in the spread of lice infestations. In the treated group which has exposure of crude extract of *C. auriculata*, there was marked reduction in physical contact between lice. After exposure to plant extract, the lice appeared disoriented and significantly reduced the lice mobility, which hindered their ability to engage with one another. In addition, the treated group which were exposed to plant extract, normally head lice formed cluster to maintain their close contact with each other, but in treated group, the lice were seen distancing themselves from one another.

These behavioural changes depicted that the plant extract may affect the nervous system possibly inducing a state of distress that drove them to avoid interactions. The reduction of physical contact between lice by plant extract exposer has been an important implication for lice management, as it could be reducing the spread of infestations. In control group, lice showed a typical social behaviour, with often contact and fast mobility across the Petri dish. These stark contrasts further emphasize the effectiveness of the plant extract against the pest management especially against head lice infestation. Due to the effects of plant extract, the lice underwent behavioural changes and were seeking refuge from the toxic effects of the plant extract.

The corners of the Petri dishes likely represented a perceived "safe zone" for the lice, though this behaviour failed to protect them from the lethal effects of plant extracts. The reduced mobility and isolating themselves and moving towards the corner of Petri dishes indicated the breakdown in the lice's ability to function normally, which can be associated with neurotoxic effects of bioactive compounds present in the plant extract. In contrast, the control group exhibited the normal social behaviours and typical distribution patterns across the Petri dish and showed faster mobility and clustering behaviours and no signs of isolation or distress. Similar findings were reported in the treatment of *Azadirachta indica* and *Melaleuca alternifolia*. Both plants extracts show significant anti-lice activity due to their high terpene and phenolic content. These compounds are effectively disrupting the respiratory function of lice.[8,22]

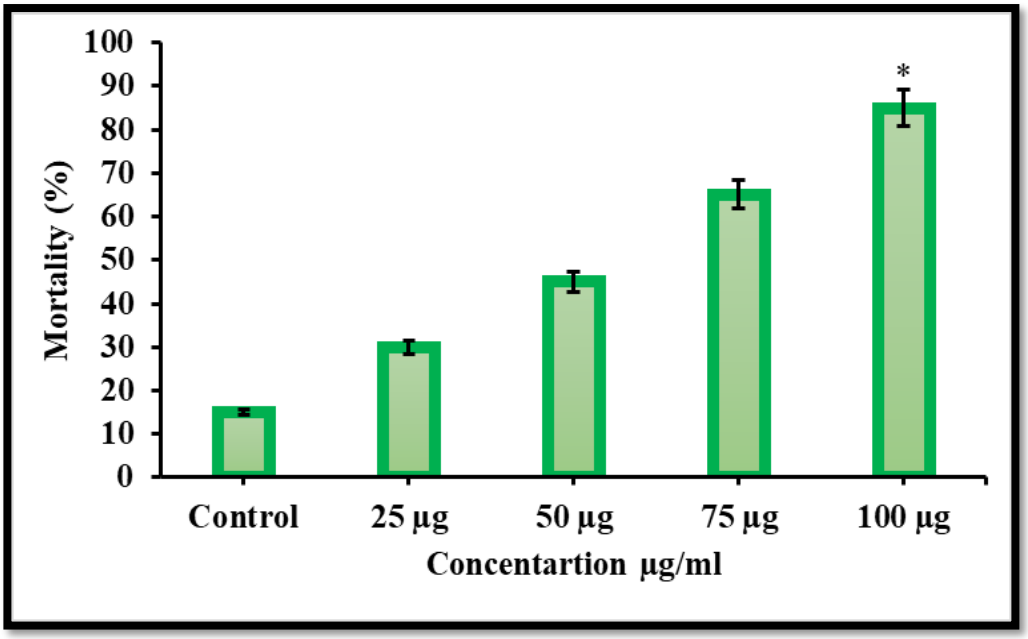

Figure 3: Contact toxicity of crude *C. auriculata* flower extract at different concentrations (25, 50, 75 and 100 µg/ml) against head lice.

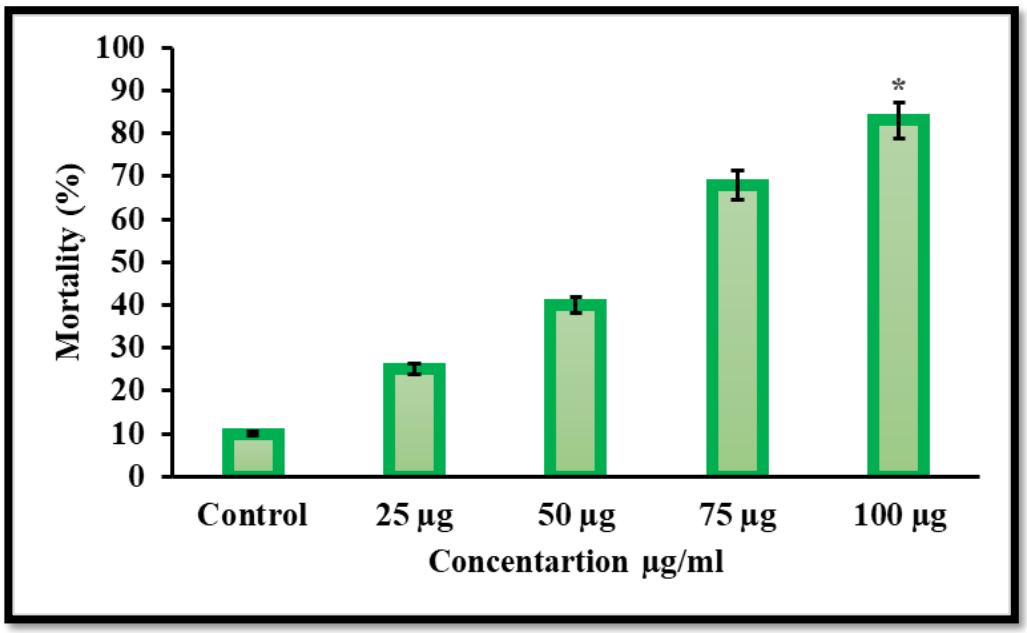

Figure 4: Acute dermal toxicity of crude *C. auriculata* flower extract at different concentrations (25, 50, 75 and 100 µg/ml) against head lice

In comparison, *C. auriculata* outperforms as potential and effective pesticide by reducing resistance development and less toxicity to humans and environment. Furthermore, the use of crude plant extract like *C. auriculata* provides a sustainable alternative with less side effects compared to several synthetic chemical products. Thus *C. auriculata* holds potential as a natural remedy against head lice and acts as effective agent in both prevention and treatment. This further reinforced the conclusion that the behavioural changes observed in the treated group were directly attributable to the effects of the *C. auriculata* flower extract.

Acute dermal irritation/corrosion assay: In the experiment, varying concentrations of crude flower extract of *C. auriculata* were applied to isolated head lice populations to observe mortality rates over a controlled period. These findings clearly depicted that the mortality rate of lice is increased when the concentration of *C. auriculata* flower extract increased. This finding is consistent with the hypothesis that bioactive compounds in the flower extract, possibly alkaloids, flavonoids, or tannins, possess strong insecticidal properties. These compounds disrupt the nervous system or the protective outer layer of the lice, leading to paralysis and eventual death. Head lice showed 80% mortality in 100 µg/ml concentration of crude flower extract of *C. auriculata*. As the concentration of *C. auriculata* flower extract increases, the rate of mortality also increases simultaneously.

Meanwhile, the control lice did not express any physical changes or mortality (Figure 4). The assay like acute dermal irritation/corrosion assay was performed to ensure that the treatment was administered in a safe and controlled manner. This assay evaluates the skin irritation potential of chemical products applied to the skin, making it an effective method to ensure that the crude extract does not cause any harm to human and environment. The head lice were exposed to various concentrations of plant extract, after certain periods of incubation, their physical condition were monitored throughout the incubation period. In addition, the control group lice population were maintained in the same condition, except the exposure of plant extract. In the control group, distilled water was used.

There are no signs of changes in physical activity of lice, or mortality was not noticed in the control group. Whereas the treated group shows the abnormal behaviour due to the bioactive components present in the *C. auriculata* flower extract. The control group shows typical behaviours including normal mobility, feeding and survival rates which suggested that the *C. auriculata* flower extract shows the efficacy in the treatment against head lice. The acute dermal irritation assay also confirmed that the *C. auriculata* flower extract in various concentrations does not cause any irritation to the skin or other biological tissues involved in the study, which demonstrated that the plant extract increased the mortality rate on increasing the concentration.

At lower concentrations, the extract displayed a slower onset of action, with some lice showing delayed mortality or reduced physical activity before eventually succumbing to the treatment. However, the higher concentration of the plant extract shows rapid immobilization followed by death within hours of exposures. These findings depicted that the flower extract acts in a dose-dependent manner, with increased concentration leading to more immediate lethal effects on the parasite. The precise mechanism by which the extract affects the lice could involves neurotoxicity, where the bioactive compounds interfere with the lice's nervous system which leads to paralysis.

Alternatively, the extract could compromise the lice's cuticle (outer protective layer), causing desiccation or dehydration, eventually leading to death. Interestingly, no adverse effects were observed in the surrounding environment or other organisms during the course of the experiment. The acute dermal irritation/corrosion assay also ensured that the flower extract did not cause irritation to the skin of the host, further supporting the use of this extract as a potential lice treatment. Unlike synthetic chemical treatments, which may cause allergic reactions, itching, or burning sensations on the scalp, the *C. auriculata* flower extract demonstrated a non-irritating and non-corrosive nature, making it a promising candidate for topical application.

The natural origin of the extract also implies a lower risk of chemical resistance, a significant advantage over conventional lice treatments that are increasingly becoming ineffective due to resistance development. In addition to the obtained results, *Azadirachta indica* (Neem) has been reported to show anti-parasitic activity, including against head lice, due to its bioactive compounds like azadirachtin, which disrupts the life cycle of lice without causing significant skin irritation.[8] Similarly, *Rosmarinus officinalis* (Rosemary) extract has exhibited insecticidal properties, with carnosic acid and rosmarinic acid contributing to lice mortality while being gentle on human skin.[34] Studies on *Eucalyptus globulus* (Eucalyptus) essential oil have shown strong pediculicidal effects, where the high concentration of 1,8-cineole plays a key role and has been found to cause minimal irritation.

Lavandula angustifolia (Lavender) oil is another plant extract known for its efficacy against lice, with linalool and linalyl acetate as primary active components that are safe for skin application. Additionally, *Citrus limon* (Lemon) peel extract has demonstrated potential lice-killing activity due to the presence of limonene and studies suggest that its application through dermal routes causes negligible irritation.[8,34]. These findings highlight the potential of plant extracts in developing natural, safe alternatives for head lice treatment.

Morphological changes: Experimental evidence has revealed that crude flower extract of *C. auriculata* induces distinct morphological changes in head lice, suggesting that it could serve as an effective natural treatment. After exposure to the plant extract, head lice showed significant changes in physiological behaviour. After exposure to plant extract, the head lice showed outward shrinkage, minor reduction and stiffness in the dorsal side and complete contraction in the whole-body. The lice treated with crude flower extract of *C. auriculata* exhibited outward shrinkage an important morphological alteration, which was evident in the overall body structure of the lice. These morphological changes depicted that plant extract was effectively involved in the reduction of body fluid content or turgor pressure within the lice due to the presence of bioactive compounds in the *C. auriculata* extract. In addition to the body shrinkage, the minor body size changes were also noticed, after exposure it increased the stiffness of the body particularly in the dorsal side. The treatment exposure in the lice increased the chance of exposure to the dorsal side which increased the appearance of rigid and inflexible and marked contrast of the supple and mobility of control group. In addition, the treatment group leads to complete contraction of the louses of the body. This contraction appeared primarily in thorax and abdominal regions which suggested that the extract not only affected the external structure but it also impacted their internal system, such as interfering with vital physiological processes including respiration and digestion.

The microscopic examination after exposure to plant extract revealed that the lice had lost much of normal anatomical flexibility with dehydrated exoskeleton and devoid of the typical elasticity seen in healthy lice. The markable changes in the body shrinkage and stiffness after exposure to the flower extract clearly depicted which interferes the lice's cuticular integrity, leading to desiccation and dehydration. Similar findings were reported in previous studies on plant-based insecticides, where similar morphological changes have been attributed to damage to the insect's cuticle, which leads to the loss of body fluid and eventual death.

Another possible mode of action of plant extract is its effectively involvement in damaging the nervous system. Most of the medicinal plants have alkaloids as major bioactive compounds and secondary metabolites, well known neurotoxins effectively disrupting the normal functioning of insect nerve cells. The stiffness of dorsal side, along with the contraction of the whole body indicated the neuromuscular disruption, which may render the lice incapable of movement of feeding, ultimately leading to their death. However, further research is needed to clarify the basic mechanism behind the action of plant extract and morphological evidence strongly supports the notion that *C. auriculata* flower extract exerts its insecticidal effects via multiple pathways.

In contrast, the control group of lice showed no significant alterations. Lice in this group retained their normal size, mobility, body shape and flexibility throughout the experiment. The dorsal side remains soft and pliable with no signs of body contraction and stiffness. These lice continued to exhibit typical behaviours, such as mobility and feeding, indicating that their physiological systems were unaffected. The markable difference between the treated and control groups demonstrated the effectiveness of *C. auriculata* flower extract as a potent anti-lice agent. The lack of remarkable changes in the control group further confirms that the alteration body parts in the treated lice were not a result of other experimental conditions but were directly caused by the exposure of the crude extract.

The lice in the control group did not exhibit any signs of distress or abnormalities indicating that extracts effects are

not related to external stress factors such as temperature, humidity, or handing. This result correlates with previous reports as *A. indica* (neem) extract has been shown to interrupt the reproductive capacity and growth of head lice and leads the physiological changes including their body size and shape and leads increased mortality rates. Similar results were reported in *R. officinalis* (rosemary), which exhibits insecticidal properties inducing significant physical alternation in lice, including a reduction in mobility and body coloration, which indicate stress and potential death.[22]

The study carried out with *Citrus limon* (lemon) extract has been reported to cause morphological deformities in lice including abnormal development of their exoskeleton, which contributes to increased rate of mortality.

In another study, the essential oil extracted form *Melaleuca alternifolia* (tea tree) showed markable effects on the respiratory system of head lice and also leads to invisible swelling and asphyxiation. The extract from *Allium sativum* (garlic) induces the changes in the structural integrity of the cuticle and leads them to lose their ability to adhere to hair shafts. All these studies collectively demonstrated that the plant extract shows the significant anti-lice property and may act as alternative treatments for head lice, also causing notable morphological changes that affects lice viability.[8,22,34] These findings highlight that the medicinal plants extracts are effective and alternative to chemical insecticides for preventing and treatment of head lice infestation.

Conclusion

The present study highlights the potential effects of *C. auriculata* against head lice. Several reports have been reported the pharmacological effects. The current study suggests that the *C. auriculata* may be used as natural alternative for managing *Pediculosis humanus capitis* infestations, a serious public health concern. The bioactive compounds present in *C. auriculata* demonstrated the effective pediculicidal activity, suggesting its safe and eco-friendly treatment option. Several studies reported that the usage of chemical agents increase the resistance of head lice, but the application of plant-based formulation offers a promising solution to address this problem sustainably.

Further research like isolating and characterizing the bioactive compounds responsible for anti-lice and evaluating their mechanisms through *in vivo* studies is needed. The scaling up the application of plant extract via clinical trials and formulation can pave the way for the commercial use of *C. auriculata*-based products, promoting to enhanced public health outcomes.

Acknowledgement

The author extends the appreciation to the Deanship of Postgraduate Studies and Scientific Research at Majmaah University for funding this research work through the project number R-2025-1905.

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(Received 21st June 2025, accepted 19th July 2025)