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Anti-lice activity of *Citrullus colocynthis* fruits against *Pediculus humanus capitis* *in vitro*

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Abstract

The increasing resistance of head lice *Pediculus humanus capitis* to many drugs has highlighted the need for new alternatives to control head lice in adults. The effect of two types of extracts (aqueous and alcoholic) of *Citrullus colocynthis* fruit on adult lice was tested *in vitro*. The results showed that the alcoholic extract with a concentration of 20% showed similar efficacy in killing adult lice to that of Natroba 9% w/w, with values ranging between 87% to 98% within 18 minutes, followed by a 20% aqueous extract with a 44% to 79% death rate. A 10% concentration of both types of extracts had moderate lethality for lice, while a 5% concentration did not show strong lethality for adult lice. These results revealed significant differences between the control group and those treated with alcoholic and aqueous extract concentrations of *C. colocynthis* fruits at the probability level $p \leq 0.05$.

Keywords: anti-lice; *Citrullus colocynthis*; *Pediculus humanus*; Diwaniyah; Iraq

Introduction

Pediculus humanus capitis, also called head lice, is a permanent ectoparasite that spends its life on one host and is characterized as a small, wingless insect that invades the hair and scalp. The infection is transmitted through contact, perhaps using shared combs, brushes, hats, or other personal items. Head lice are most common among girls aged 5 to 11 years. The emergence of head lice resistance to synthetic compounds has prompted the search for new alternatives to synthetic ingredients, most notably plant ingredients [1–3].

Citrullus colocynthis belongs to Cucurbitaceae, and is native to North Africa, southern Europe, southern Asia, and the Middle East. It grows in sandy soils in regions with high temperatures and little rainfall. *C. colocynthis* is an evergreen perennial plant that reaches three meters in height and has fruit similar to watermelon but smaller. The plant has a tough texture and a bitter-tasting pulp [2]. Many scientific sources indicate the use of different plant parts to treat many diseases, such as breast inflammation and joint pain. Externally, it was used for conjunctivitis and uterine pain [4]. The fruit has been used in cases of dropsy, yellow, jaundice, brain congestion, colic, fever, worms, and sciatica, and as a cause of abortion and to treat constipation, bacterial infections, and cancer [5]. The seed oil was used for poisonous bites, enteritis, epilepsy, and lightening hair [6,7].

The plant extracts of *C. colocynthis* fruits had a lethal effect on some insects, such as *Aedes aegypti* L., *Culex quinquefasciatus*, and *Aphis craccivora* [8,9]. There-

fore, this study examined the effects of the aqueous extract of *C. colocynthis* fruit on adult lice.

Materials and Methods

Preparation of aqueous and alcoholic extract of *C. colocynthis* fruits

The *C. colocynthis* fruit was obtained from the local markets in Al-Diwaniyah and identified by Dr. Suhaila Hussein Al-Lami, Department of Biology, Faculty of Education, University of Al-Qadisiyah. The dry fruits were ground using an electric grinder. The powder was stored in airtight glass bottles at a temperature of 4°C until use.

Fifty grams of *C. colocynthis* fruit powder was mixed with 500 mL and distilled water, 500 mL of ethyl alcohol. The mixture was placed on a magnetic stirrer for 24 hours at laboratory temperature (25°C).

The filtrate was separated from the sediment by centrifuge at 3,000 rpm for 15 minutes. The filtrate was concentrated using a rotary evaporator, and the filtrate was placed in a Petri dish, left at laboratory temperature until dried, and kept in a refrigerator [10].

Lice samples collection

Adult *P. humanus capitis* insects were collected from children aged 6 to 12 years by combing the scalp using a clean, fine-toothed comb. The lice were removed from the comb teeth and placed in plastic boxes. The children had not taken anti-lice medication for at least three months.

Anti-lice activity

After collecting the lice samples, they were examined under a dissecting microscope. An equal number of lice were distributed on 10 Petri dishes. The extracts were dissolved in distilled water to obtain three different concentrations (5%, 10%, and 20%). For the first Petri dish, 0.5 mL of distilled water was added as a control group, while plates 2 to 7 received 0.5 mL of the three concentrations of the alcoholic and aqueous extract, respectively, plates 8 to 10 were treated with 0.5 mL of Natroba 9% w/w at three concentrations.

The above groups were placed in a dark room at a temperature of 26°C ± 0.5°C and a humidity of 70% ± 1% [11]. After one hour, the Petri dishes were removed; 0.5 mL of the distilled water was added and returned to their place. After 18 minutes, the Petri dishes were examined under a microscope to observe any expected movement. The immobile lice were considered dead [12]. The dishes were checked after 3 to 6 hours to ensure the lice were dead. All treatments were repeated three times.

Ethical approval

All procedures were conducted in accordance with the ethical standards of the institution at which the studies were conducted. The current study was approved by the Committee of the Department of Biology, Faculty of Education, University of AL-Qadisiyah.

Results

A 20% concentration of the aqueous and alcoholic extract of the fruit of the *C. colocynthis* plant led to the inhibition and paralysis of louse movement and caused death at shorter times than the control group (Table 1), which were shorter than in the case of the control group.

At higher concentrations, the time required to paralyze tick movement and cause death was shorter. The 20% alcoholic extract was the most efficient in eliminating lice compared to the Natroba drug because it led to the immobilization of lice and caused 98% death, followed by the 20% aqueous extract, which paralyzed the movement of lice and resulted in a 79% death rates.

The 10% aqueous and alcoholic extract led to 44% and 87% lethality of lice, respectively. By contrast, the mortality rate ranged between 20% and 48% for the 5% aqueous and alcoholic extracts, respectively. The results indicated significant differences between the control group and treated groups at the $p \leq 0.05$ level.

Discussion

Lice in many geographical areas have acquired resistance to some available drugs, possibly because of their incorrect use, such as not repeating the treatment on time. Many studies indi-

Table 1. Effect of the *Citrullus colocynthis* fruit extracts on adult head lice mortality

Test samples	Concentration	Mortality rate
Distilled water 0.5 mL	-	8.92 ± 0.04
Water extract of <i>C. colocynthis</i> 0.5 mL	5	20 ± 0.13
	10	44 ± 0.52
	20	79 ± 3.91
Alcoholic extract of <i>C. colocynthis</i> 0.5 mL	10	48 ± 4.66
	20	87 ± 5.14
Natroba 9% w/w (0.5 mL)		98 ± 4.44
	5	60 ± 4.78
	10	94 ± 3.27
	20	100 ± 1.83

Mortality rates are presented as mean ± standard deviation. -, without concentration.

cated that plant extracts, such as eucalyptus, marjoram, spearmint, peppermint, sage, rosewood, clove bud, cinnamon bark, custard apple seeds, and *Pongamia pinnata* seed, had a lice-repellent effect [13–15].

The current study showed that the alcoholic and aqueous extracts of the *C. colocynthis* fruit have a clear effect on adult lice death, possibly because the *C. colocynthis* fruit contain many effective compounds, such as carbohydrates, proteins, separated amino acid, tannins, saponins, phenolics, flavanoids, flavone glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, cardiac glycoloids, trace elements and many other chemical groups [16]. This effect was directly proportional to the concentration and duration of exposure to these extracts. In addition, the percentage of adult lice deaths differed according to the type of extract.

The alcoholic extract of the *C. colocynthis* fruit, which was used to study the effectiveness of this extract against adult lice, led to the inhibition and paralysis of tick movement and then its death after different periods. This may be because this extract contains many active substances that come into direct contact with the lice body, leading to their death in a short period, or because the nervous and digestive systems of the insect are affected by contact with this extract, or these substances enter through the breathing holes, which leads to suffocation [17]. These results are in agreement with the researchers' findings [8,9], who confirmed that the plant extracts of *C. colocynthis* have a fatal effect on some insects, such as *A. craccivora*, *Aedes aegypti* L., and *C. quinquefasciatus*. A previous study used the plant extracts from the *C. colocynthis* fruit to treat cattle ticks because they contain alkaloids, pectic compounds, and soapy substances [18].

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