ABSTRACT
Head lice, *Pediculus humanus capitis* are obligatory ecto-parasites on humans all over the world. They cannot be transmitted to or from pets or other animals due to extremely host-specific. The three effectual essential treatment options for head lice are wet combing, topical pediculicides and oral therapy. Some botanical extracts are assumed obvious pediculicidal activity in different exposure periods. In present study, *Allium cepa* (Onion bulb juice), *Cucumis sativus* (Cucumber fruit juice) and Camel urine were used concentrated (100%) in vitro assessment by applying new method named as filter paper diffusion bioassay. All the concentrated tested materials used in this trial showed low activity against *Pediculus humanus capitis*. The mean of head lice mortality was 10% and 20% by using the Onion juice after 30 and 60 minutes and Cucumber juice for 60 minutes respectively, while the head lice survival rate was 100% by using the Camel urine as pediculicidal agent. The pediculicidal activity of Camel urine, the fully concentrated juice of Cucumber fruit and Onion bulb was limited in this designed exposure periods which mean these substances should be chemically extracted and tested for their pediculicidal action as concentrated extracts or as active components by using a new experimental technique.

KEYWORDS: Head Lice, Pediculicidal Activity, Onion Bulb Juice, Cucumber Fruit Juice, Camel Urine, Filter Paper Diffusion Bioassay.

INTRODUCTION
Almost five million children between six and twelve years old each year have the head lice infection (Gratz, 1997; Scanni and Bonifazi, 2008). In many developed countries, *pediculosis capitis* increased in the last two decades, despite the enhanced hygienic situation and all the head lice treatment achieved (Burgess, 2004; Kim et al., 2004).

The most effective procedure for elimination *pediculosis capitis* is to use an efficient treatment as a pediculicide that kill head lice and their nits, followed by the manual nit removal (Meinking, 1999; Frankowski and Weiner, 2002; Frankowski, 2004; Burgess, 2006). The herbal therapy based or origin bioactive molecules derived from cardamom ceylon,
clove bud, myrtle, rosewood, eucalyptus, tea tree, neem, lavender, geranium and several other plants (Heukelbach et al., 2008).

Shrivastava et al. (2010) quoted that *Citrus limon* has been used for both cooking and therapeutic purposes. To check the pediculicidal activity of *Citrus limon* juice, it was examined *in vitro* toxicity model which observed for response lacking to stimuli over three hour as exposure period.

*Allium cepa* is highly valued for its therapeutic properties and recognized as an important medicinal plant for many years ago. Recently, Onion extracts reported to be effective as anticardiovascular, antihypertensive and antidiabetic by few workers. It possesses many other biological activities including antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic etc (Corzo-Martínez et al., 2007). Saleheen et al. (2004) studied the effect of aqueous onion extract, as antileishmanial *in vitro*, the IC$_{100}$ was 1.25 mg/ml and the IC$_{50}$ value was 0.376 mg/ml against the leishmanial strain tested. The antimicrobial activity of different concentrations of essential oil extracts of three type of onions (green, yellow and red) were tested against two bacteria, *Staphylococcus aureus*, *Salmonella enteritidis*, and three fungi, *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum* by Benkeblia (2004). The essential oil extracts exhibited marked antibacterial activity showing the lowest inhibition in green onion while *A. niger* and *P. cyclopium* were significantly inhibited particularly at low concentrations of these extracts. The results of using *Allium cepa* oil on the development and progression of the experimental cryptosporidiosis in mice showed that the administration of onion oil was beneficial in protecting susceptible hosts against *Cryptosporidium parvum* (Abu El Ezz et al., 2011).

The evaluation of the *Cucumis sativus* aqueous and ethanol extracts against Indian adult earthworms, *Pheritima posthuma* was done by Kumar et al., 2010b, a concentration of 10 mg/ml of albendazole was used as standard reference. *Cucumis sativus* ethanol extracts showed paralysis and death time in 4.12 and 6.58 minutes respectively at a concentration of 50mg/ml, which was better than the *Cucumis sativus* aqueous extract.

The Camel urine is proficient in the treatment of human skin and hair diseases as well as it is good in removing the scalp dandruff and changing the human hair to be thick and shiny (Havnes, 1898). Jannah et al. (2013) reported that the Camel urine bio-active fraction which was coded as PMF was capable considerably to feed the normal skin fibroblast. This fraction was formulated as ointment (the base composed of white soft paraffin 95% w/w, white bees wax 5% w/w and appropriate preservatives) to treat many skin diseases. In one case of a boy
as 18 years old who was having psoriasis on the scalp and neck back, the treatment using PMF ointment showed good result as an anti proliferative agent.

The aim of this study is to conduct an in vitro assessment for the pediculicidal activity of some natural materials against the *Pediculus humanus capitis* comparing with Licid shampoo, the trade chemical product, by applying new method named as filter paper diffusion bioassay in a period of maximum three hours to avoid the starving effect of head lice.

**MATERIALS AND METHODS**

**Specimens Collection:**

Human head lice were collected from the infected girls students in the elementary schools, between the age of 7-12 years in a carefully manner. It is very important to ensure that the student has not undergone the head lice treatment, either natural treatment or chemical one. The student hair was combed by fine dry lice comb under the approval of her schoolteacher or administrator. The head lice were collected by putting a white paper sheet under the head of the student, so that nearly all head lice would have fallen on the paper during the hair combing. The collected head lice were put in plastic boxes with lids, and transferred to the laboratory immediately after collection.

**Materials Description:**

*Allium cepa*, Onion bulb is a tunicated, compressed or round bulb, or oblong in figure, invested with a shining, thin, dry membrane, of a reddish or white color. It is less pungent to the taste than garlic, with some degree of sweetness, and a peculiar well-known odor. Onion bulbs are of various shapes and sizes, usually globular, the layers being juicy (Kumar et al. 2010a).  *Cucumis sativus*, Cucumber fruit is an extensively cultivated plant in the family Cucurbitaceae. It is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around ribbing with thin and spiraling tendrils. The plant has large leaves that form a canopy over the fruit. The fruit is roughly cylindrical, elongated, with tapered ends (Kumar et al. 2010b). Camel urine is collected from the young Camel in the early morning. It was put in a clean bottle and transferred immediately to the laboratory for using fresh as an anti-head lice agent.

**Materials Preparation:**

The fresh Onion bulbs were peeled and squeezed by juicer fruits and the same procedure was done with Cucumber fruit. These materials as well as Camel urine were used as 100% concentrated and fresh for testing their activity in killing the human head lice.
Reference Insecticide:

Licid shampoo was used in this study as a positive control. The active ingredient of Licid shampoo are bioallethrin and Piperonyl butoxide, it also consists of a collection of oils (Olive oil, Chamomile oil, Anise oil and Cactus oil). This shampoo was brought from one of the Saudi Health Center, which is located near one of the public schools in Jeddah city/Saudi Arabia. It was distributed free for the girl students infested with head lice.

Testing for Anti-pediculosis Activity:

To evaluate the activity of the tested materials as antipediculosis, the protocol of the experiments was conducted in the room temperature of 29 ± 0.5 °C and the humidity was of 70 ± 1%. Three experimental replicates using groups of human head lice were conducted to investigate the antipediculosis activity of the tested substances as concentrated materials without dilutions.

The *P. humanus capitis* were collected from the infested student hairs on the same day the test was conducted (approximately from 1-4 hours as a maximum period) to avoid the effect of head lice starving. The *in vitro* tests were almost started within one hour after collection. A filter paper diffusion bioassay was made according to Shrivastava et al. (2010). After a careful selection of head lice under a dissecting microscope, filter papers discs (Whatman No 1) were cut and placed in the bottom of Petri dishes. 10 head lice (adults and nymphs) were placed in every filter paper and then 1 ml of each substance was spread over the head lice and filter paper. The head lice as dead or alive were observed at four intervals of 30, 60, 90 minutes and 3 hours. The test was done in triplicate and the average number of dead and alive lice was considered. If there were no symptoms of activity shown on the head lice (upon stimulation with a forceps), they were considered dead.

Two groups of control were conducted following the same procedure, negative control and positive control. The negative control head lice were placed in the same size of filter paper disc in a Petri dish and immersed only by using distilled water, while the positive control was conducted by using the Licid shampoo.

RESULT AND DISCUSSION

The mortality rate of head lice (adult and nymph) was evaluated after treatment with Onion bulb (*Allium cepa*), Cucumber fruit (*Cucumis sativus*) and Camel urine for a maximum
period of three hours, to avoid the head lice starving effect, and compared with the group of positive and negative controls.

The results showed that Onion bulb juice, *Allium cepa*, Cucumber fruit juice, *Cucumis sativus* and urine of young Camel had limited effect that the head lice movement was observed on most head lice even after the maximum exposure period of three hours (Table 1, Figure 1). In one of the Onion triplicate tests, three head lice stopped moving in a period of one hour, but they returned to move after 90 minutes. The same action was happened by using the Cucumber juice that six head lice stopped crawling at 60 minutes in two replicates, but they became active at 90 minutes. The mean death was recorded by using the Onion juice as one head lice after 30 to 60 minutes, giving the mortality percentage as 10%, while the mean mortality was two insects when the head lice exposed to the Cucumber juice for 60 minutes and the mortality percentage was 20%. The Camel urine showed no activity in stopping the crawling of human head lice, that the survival percentage was 100%.

Table (1): The effect of *Allium cepa* juice, *Cucumis sativus* juice, Camel urine and Licid shampoo against the adult head lice, *Pediculus humanus capitis* after exposing to the tested materials for 30, 60, 90 minutes and 3 hours.

By using the Licid shampoo as a positive control, the mean of head lice mortality was found as 10 head lice in the period of 30 to 180 minutes, while all ecto-parasite insects exposed only to the distilled water showed active movement even after three hours as a maximum exposure time. The pediculosis research experiments and the predictions for new and ecologically
tolerable pediculicide are still in progress. Currently filter paper tests in Petri dishes are communal bioassay technique to define the level of topical pediculicide resistance in *Pediculus humanus capitis* which affords informative and comparable results. Onion bulb juice, Cucumber fruit juice and Camel urine showed less activity than other results recorded by Carpinella et al. (2007), who recorded that 70.1% of the head lice were killed by using 20% from fruits of *Melia azedarach*, 90.1% by 20% ripe fruit oil, 96.5% by 20% ripe fruit extract + ripe fruit oil, and finally 92.3% by using the formulation of 10% ripe fruit extract + ripe fruit oil + emulsifier and preserving agents in an exposure period of 4 hours. Also it is lower than that mentioned by Surendra et al. (2013), who declared that 86.66% from *Pediculus humanus capitis* was exposed to death in 18 hours by using 25% alcoholic extract of *Myristica fragrans* Houtt fruit pulp. Samuel et al. (2009) stated that 100% from the head lice were killed in 18 hours by using 20% petroleum ether extract of *Pongamia pinnata* leaves, and Sherwani et al. (2013) documented that 100% of head lice killed in 18 hours by using 20% *Camellia sinensis* and green tea, these findings are higher than the result of tested materials reported here because the exposure periods in the experiments of Surendra et al. (2013), Samuel et al. (2009) and Sherwani et al. (2013) were very long and the head lice exposed to the starving effect. The *Citrus limon* juice and ½ diluted juice showed 95±5% and 90±10% respectively as pediculcidal substances (Shrivastava et al., 2010) was better than the results of Onion juice and Cucumber juice reported here with considering that all the tested materials conducted at the same exposure periods of maximum three hours. The present study of Camel urine examination effect as antipediculosis substance is considered the first one in this area of search and was not mentioned before, although the activity of Camel urine in killing the head lice had limited effect by using the experiment design mentioned here.

![Figure (1): The effect of *Allium cepa* juice, *Cucumis sativus* juice, Camel urine and Licid shampoo against the adult head lice, *Pediculus humanus capitis* after exposing to the tested materials for 30, 60, 90 and 3 hours](image)

In conclusion the three tested materials as pediculcidal agents are not active in the experimental pattern followed in this study, which mean that the substances should be used as...
chemical concentrated extracts and their active components should be identified and examined as pediculosis agent that may showed an encouraging result in the future. The experiment design should be developed or the exposure time should be added to ensure the pediculicidal action is completed.

REFERENCES