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To study the pediculocidal activity of *Euphorbia helioscopia*, *Sapium sabiferum* and *Callistemon citrin* against *Pediculus humanus capitis*

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Key words: *Pediculus humanus capitis*, anti-lice activity, plant extract, *Euphorbia helioscopia*, *Sapium sabiferum* and *Callistemon citrinus*.

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Abstract

Human head and clothing lice are wildly present infesting millions of school children every year. Insecticides such as DDT, lindane, malathion, carbaryl, permethrin and d-phenothrin has been used for decades. Recently, scientists take great interest in the use of plant extracts as a new control alternative to synthetic insecticides. In present study, antilice potential of three local plants, *Euphorbia helioscopia*, *Sapium sabiferum* and *Callistemon citrinus* against *Pediculus humanus capitis* was determine. Plant samples were collected from territory of Quaid-i-Azam University, Islamabad. For determination of pediculocidal of these plants, 40 lice were placed in each petri dish having filter paper soaked in different concentrations (100ppm, 200ppm, 300ppm) of water and methanol extracts of leaves. In many choice experiment percent mortality of lice was calculated after 6, 12 and 24 hrs. Maximum mortality rate of *E. helioscopia*, *S. sabiferum* and *C. citrinus* was observed after 24 hours for all three plants in water solvent (9.67, 12.67, 14.67% and 6.34, 8.67, 12.00% and 0.00, 0.33, and 0.66% respectively) as well as in methanol solvent (13.67, 14.33 and 16.67%, 3.67, 6.00 and 1.93%, 0.34, 0.66 and 1.00% respectively). Among three solvent extracts tested, the maximum activity was observed in both water and methanolic extracts of *E. helioscopia* against *P. humanus capitis*.

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Introduction

Human head and clothing lice are found in every continent. Head lice are common worldwide and infesting millions of school children every year. This resistance is due to the evolution of resistance to insecticidal shampoos that are used to treat the infestation of pediculosis (Burgess, 1995). The estimation suggested that 5 million people are newly infested with head lice each year and these parasites mostly affect children who are 6 to 12 years old (Gratz, 2006). Their presence may cause itching, loss of sleep and secondary skin infections due to scratching of irritated area (Gratz, 2006; Durden, 2005). Insecticides such as DDT, lindane, malathion, carbaryl, permethrin and d-phenothrin has been used for decades, with continued or repeated application through which control has been made. Resistance has been developed due to the repeated use of these insecticides in several countries (Picollo *et al.*, 1998; Burgess, 2004). Pediculosis has increased worldwide as a result of insecticide failures through resistance, improper application, formulation changes and misdiagnosis, during last two decades (Burgess, 2004; Kim *et al.*, 2004). Recently, scientists take great interest in the use of plant extracts as a new control alternative to synthetic insecticides. Essential oils seem to be good replacer of synthetic insecticides, as many have lesser mammalian toxicity and lesser persistence in the environment than synthetic insecticides (Isman, 1999). Some of these natural insecticides are effective against a variety of insect pests (Isman, 1999; Lee *et al.*, 2003), including *Pediculus humanus capitis* (Mumcuoglu *et al.*, 1996). By steam distillation of plant tissues (either wild or cultivated plants) majority of essential oils are obtained and consist of mixtures of hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers) (Guenther, 1972). A plant's distinctive scent is often depended on these oils. An estimation demonstrated that 3000 essential oils are known, of which 300 are of commercial importance (FAO, 1995). In developing countries lice infestations are even more common, as synthetic pediculicides are either unavailable or prohibitively expensive cures for pediculosis center mainly on social grooming (Toups, 2008.). Constituents of plant volatile oils affect the behavioral responses of pests, with the monoterpenoid components that appear most useful as insecticides or anti feedants (Palevitch and Crake, 1994). Essential oil constituents are reputed to have good ovicidal capabilities, as many pediculicides failed due to low efficacy on lice egg (Burgess, 2004). It was also determined whether a single compound could suffice in a pediculicidal formulation, or a combination of agents would be significantly better. Essential oils have often been proposed as alternative pediculosis control agents and their constituents therefore provide a good starting point for an investigation into the development of novel pediculicides. Attempt of botanical insecticides, pesticides and pediculocides have been in practice since long in some form or the other for controlling insects **(**Ramdev and Sharma, 2011). Essential oils have a wide pharmaceutical activities including anti-plasmodial (Boyom *et al.*, 2003), anti-leishmanial (Ahmed *et al.*, 2006), anti-cancer (Sylvestre *et al.*, 2009) and insecticidal (Perez-Amador *et al.*, 2003).

The aim of present study is to evaluate pediculocidal properties of *Euphorbia helioscopia, Sapium sebiferum* and *Callistemon viminallis* against *P. humanus capitis*.

Materials and methods

Samples collection:

Pediculus humanus capitis were collected by combing hair of children, locally within the locality of Quaid-i-Azam University, Islamabad, Pakistan. Live material was kept in petri plates and was used immediately after collection.

Selection of Plants

The leaves of *Euphorbia helioscopia* (Fig 1A), *Sapium sabiferum* (Fig 1B) and *Callistemon citrinus* (Fig 1C) have been collected locally within the locality of Quaid-i-Azam University, Islamabad, Pakistan and identified by using key (Table 1) (Nasir and Ali, 1977).

Extraction method

Leaves of *E. helioscopia, S. sebiferum* and *C. citrinus* were splashed with water to remove the accompanying organisms and attached salts. After that the leaves were dried in oven at 37 ºC and crumpled with the aid of electric grinder. The material selected was 40 passed and 60 mesh retained. 30g grinded leaves of the plants used for experiments were extracted in 300ml of two different solvents i.e. methanol and water for 6 to 8 hour (two cycles per hour) in a soxhelt extraction apparatus. Whattman no.1 filter paper was used to filter the extracts. The dried residues were collected by evaporating the solvent with the help of rotary vacuum evaporator and stored in a refrigerator for making stock solution.

Formulation of stock solution

One gram of the plant residue was dissolved in 100ml of distilled water (stock solution).The stock solution was further diluted with the help of dilution formula (Jayal *et al.*, 2006).

$$C_1V_1=C_2V_2\ V1 = \frac{\text{required parts par million x required volume}}{\text{Stock solution}}$$

Antilice Assay

The study was done by filter paper diffusion method (Singh *et al.*, 2011). Samples of 1, 2 and 3 ml from stock solution were dissolved in 100 ml of distilled water to make different concentrations like 100ppm, 200ppm and 300ppm respectively, and 1.5 ml of each solution was applied on filter paper (Whattman No. 1). For control group filter paper were treated with solvents (water and methanol) only. After the evaporation of solvent from filter papers by drying in air, two filter papers were placed in the bottom of each petri dish provided with lid. A soaked cotton plug was placed in each petri dish to maintain their moisture content. Twenty active lice were put into each petri dish on filter paper, covered and placed in dark. Additionally the lice was not exposed in an enclosed environment with the petri dish kept open, which limits the possibility of volatile agents getting absorbed through the spiracles. Extract was tested for pediculocidal activity by filter paper diffusion method (Picollo *et al.,* 2000). After the time interval of 6, 12 and 24 hrs, the dishes were observed under a dissecting microscope for any possible movement of lice and absence of any movement were considered dead (Meinking *et al.*, 1986).

Experiment was conducted in triplicate for each sample concentration along with the set of control and percent mortality was counted after equal time period mentioned above.

% mortality = ODP÷TP×100

Statistical analysis

Results of morphometric analysis such as mean, standard deviation and coefficient of variance were analyzed by using student "t" test to determine the variations in different parameters. Mortality ratio percentage of lice was calculated and analyzed by using one way Anova and Tukey test. Values of $P<0.05$ were considered significant statistically. After 24 hrs of exposure to each extract LC_{50} and LC_{90} was calculated by using Probit analysis (Finney, 1971).

Results

Effect of Euphorbia helioscopia leaves extract on Pediculus humanus capitis in water solvent

Water soluble leaves extract of *E. helioscopia* showed 3.34±0.34, 4.34±0.34 and 5.67±0.67 percent mortality by feeding on 100, 200 and 300ppm respectively after 6 hours of experiment where as in control mortality was also observed i.e. 2.34±0.34. LC_{50} value was 0.059% and value of LC_{90} was 0.123%. After 12 hours of experiment percent mortality rate on same concentration (100, 200 and 300ppm) was 5.67±0.67, 8.33±0.89 and 9.34±1.34 while in control observed mortality was as 9.34±1.34 which was non-significant. LC_{50} value was 0.0318% and value of LC_{90} was 0.084%. Maximum mortality rate was observed after 24 hours of experiment at all above mentioned concentrations which was 9.67±1.2, 12.67±0.89 and 14.67±0.34 and non significant mortality rate of control was 3.34±0.34 observed. LC_{50} value was 0.0107% and value of LC_{90} was 0.049% (Table 2).

Effect of Euphorbia helioscopia leaves extract on

Pediculus humanus capitis in methanol solvent

Leaves extract of *E. helioscopia* methanol solvent showed 5.33±0.58, 6.00±0.57 and 7.67±.34 percent mortality by feeding on 100, 200 and 300ppm respectively after 6 hours of experiment where as in control no significant mortality was observed i.e. 1.67±0.34. LC_{50} value was 0.04% and value of LC_{90} was 0.12%. After 12 hours of experiment percent mortality rate on same concentration (100, 200 and 300ppm) was 8.33±0.34, 10.67±0.89 and 13.67±0.89 while in control observed mortality was as 3.33±0.34 which was non-significant. LC_{50} value was 0.015% and value of LC_{90} was 0.146%. Maximum mortality rate was observed after 24 hours of experiment at all concentrations which was 13.67±0.89, 14.33±0.89 and 16.67±0.33 and mortality rate of control was 5.33±0.58 which was non-significant. LC_{50} value was 0.003% and value of LC_{90} was 0.095% (Table 3).

Table 1. Plants selected for experiments during present research.

Sr.#	Botanical name	English name	Vernacular name	Family
1	*Euphorbia helioscopia*	Sun spurge	Gandi booti	Euphorbiaceae
2	*Sapium sabiferum*	Chinese tallow tree	Popcorn Tree	Euphorbiaceae
3	*Callistemon citrinus*	Bottle brush	Bursh	Myrtaceae

Table 2. Percentage mortality of *Pediculus humanas capitis* in different concentrations of *Euphorbia helioscopia* in water.

Time (hours)	Concentrations (ppm)				LC_{50} %	LC_{90} %
	Control	100	200	300		
6	2.34±0.34a	3.34±0.34ab	4.34±0.34bc	5.67±0.67c	0.059	0.123
12	2.67±0.43a	5.67±0.67a	8.33±0.89ab	9.34±1.34b	0.031	0.084
24	3.34±0.34a	9.67±1.2ab	12.67±0.89b	14.67±0.34c	0.010	0.049

Values in the same columns with different letters are significantly different by Tukey test ($p<0.05$).

Effect of Sapium sebiferum leaves extract on Pediculus humanus capitis in water solvent

Water soluble leaves extract of *S. sebiferum is* showed 3.34±0.34, 4.67±0.34 and 6.34±0.34 percent mortality by feeding on 100, 200 and 300ppm respectively after 6 hours of experiment where as in control no significant mortality was observed i.e. 1.67±0.34. LC_{50} value was 0.049% and value of LC_{90} was 0.101%. After 12 hours of experiment percent mortality rate on same concentration (100, 200 and 300ppm) was 4.67±0.67, 6.00±0.58 and 7.67±0.34, while in control observed mortality was as 1.67±0.34 which was non-significant. LC_{50} value was 0.049% and value of LC_{90} was 0.103%. Maximum mortality rate was observed after 24 hours of experiment at all above mentioned concentrations which was 6.34±0.34, 8.67±0.67 and 12.00±1.00 and mortality rate of control was 3.67±0.34 which was not significant. LC_{50} value was 0.023 and value of LC_{90} was 0.058% (Table 4).

Effect of Sapium sebiferum leaves extract on Pediculus humanus capitis in methanol solvent

Leaves extract of *S. sebiferum is* in methanol solvent showed 2.34±0.67, 4.00±0.58 and 4.34±0.34 percent mortality by feeding on 100, 200 and 300ppm respectively after 6 hours of experiment where as in control no significant mortality was observed i.e. 0.33±0.34. LC_{50} value was 0.067% and value of LC_{90} was 0.132%. After 12 hours of experiment percent mortality rate on same concentration (100, 200 and 300ppm) was 3.33±0.89, 5.33±0.89 and 7.00±1.94 while in control observed mortality was as 1.00±0.58 which was not significant. LC_{50} value was 0.042% and value of LC_{90} was 0.087%. Maximum mortality rate was observed after 24 hours of experiment at all above mentioned concentrations which was 3.67±0.89, 6.00±0.58 and 1.93±2.18 and mortality rate of control was 1.34±0.67 which was not significant. LC_{50} value was 0.027% and value of LC_{90} was 0.051% (Table 5).

Table 3. Percentage mortality of *Pediculus humanas capitis* in different concentrations of *Euphorbia helioscopia* in methanol.

Time	Concentrations (ppm)				LC_{50} %	LC_{90} %
	Control	100	200	300		
6(hours)	1.67±0.34a	5.33±0.58a	6.00±0.57a	7.67±0.34b	0.049	0.128
12(hours)	3.33±0.34a	8.33±0.34ab	10.67±0.89b	13.67±0.89c	0.015	0.146
24(hours)	5.33±0.58a	13.67±0.89a	14.33±0.89a	16.67±0.33b	0.003	0.095

Values in the same columns with different letters are significantly different by Tukey test ($p<0.05$).

Table 4. Percentage mortality of *Pediculus humanas capitis* in different concentrations of *Sapium sebiferum* in water.

Time	Concentrations (ppm)				LC_{50}%	LC_{90}%
	Control	100	200	300		
6(hours)	1.67±0.34a	3.34±0.34b	4.67±0.34b	6.34±0.34c	0.049	0.101
12(hours)	2.34±0.34a	4.67±0.67ab	6.00±0.58b	7.67±0.34c	0.049	0.103
24(hours)	3.67±0.34a	6.34±0.89ab	8.67±0.67bc	12.0±1.00c	0.023	0.058

Values in the same columns with different letters are significantly different by Tukey test ($p<0.05$).

Effect of Callistemon citrinusle aves extract on Pediculus humanus capitis in water solvent

Water soluble leaves extract of *C. citrinus*showed 0.00±0.00, 0.00±0.00 and 0.00±0.00 percent mortality by feeding on 100, 200 and 300ppm respectively after 6 hours of experiment where as in control no mortality was observed i.e. 0.00±0.00. LC_{50} value was also 0.0% and value of LC_{90} was 0.00%. After 12 hours of experiment percent mortality rate on same concentration (100, 200 and 300ppm) was also 0.00±0.00, 0.00±0.00 and 0.00±0.00 while in control observed mortality was as 0.00±0.00 which was not significant. LC_{50} value was 0.00% and value of LC_{90} was 0.00%. Mortality rate was observed after 24 hours of experiment at all above mentioned concentrations which was 0.00±0.00, 0.33±0.34 and 0.66±0.67 and non significant mortality rate of control was 0.00±0.00 observed. LC_{50} value was 0.065% and value of LC_{90} was 0.090% (Table 6).

Table 5. Percentage mortality of *Pediculus humanas capitis* in different concentrations of *Sapium sebiferum* in methanol.

Time	Concentrations (ppm)				LC_{50}%	LC_{90} %
	Control	100	200	300		
6(hours)	0.33±0.34a	2.34±0.67a	4.00±0.58ab	4.34±0.34b	0.067	0.132
12(hours)	1.00±0.58a	3.33±0.89a	5.33±0.89a	7.00±1.94a	0.042	0.087
24(hours)	1.34±0.67a	3.67±0.89ab	6.00±0.58ab	1.93±2.18b	0.027	0.051

Values in the same columns with different letters are significantly different by Tukey test ($p<0.05$).

Table 6. Percentage mortality of *Pediculus humanus capitis* in different concentrations of *Callistemon citrinus* in water.

Time	Concentrations (ppm)				LC_{50} %	LC_{90} %
	Control	100	200	300		
6(hours)	0.00±0.00a	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00	0.00
12(hours)	0.00±0.00a	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00	0.00
24(hours)	0.00±0.00a	0.00±0.00a	0.33±0.34a	0.66±0.67a	0.065	0.090

Values in the same columns with different letters are significantly different by Tukey test ($p<0.05$).

Effect of Callistemon citrinus leaves extract on Pediculus humanus capitis in methanol solvent

Water soluble leaves extract of *C. citrinus* showed 0.00±0.00, 0.00±0.00 and 0.00±0.00 percent mortality by feeding on 100, 200 and 300ppm respectively. After 6 hours of experiment where as in control no mortality was observed i.e. 0.00±0.00. LC_{50} value was also 0.0% and value of LC_{90} was 0.00%. After 12 hours of experiment percent mortality rate on same concentration (100, 200 and 300ppm) was also 0.00±0.00, 0.00±0.00 and 0.00±0.00 while in control observed mortality was as 0.00±0.00 which was not significant. LC_{50} value was 0.00% and value of LC_{90} was 0.00%. Mortality rate was observed after 24 hours of experiment at all above mentioned concentrations which was 0.00±0.00, 0.34±0.34 and 0.66±0.67 and non significant mortality rate of control was 0.00±0.00 observed. LC_{50} value was 0.099% and value of LC_{90} was 0.154% (Table 7).

Table 7. Percentage mortality of *Pediculus humanus capitis* in different concentrations of *Callistemon citrinus* in methanol.

Time	Concentrations (ppm)				LC_{50} %	LC_{90} %
	Control	100	200	300		
6(hours)	0.00±0.00a	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00	0.00
12(hours)	0.00±0.00a	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00	0.00
24(hours)	0.00±0.00a	0.34±0.34a	0.66±0.67a	1.00±0.34a	0.099	0.154

Values in the same columns with different letters are significantly different by Tukey test ($p<0.05$).

Discussion

Lice infestations are common worldwide whereas most of the currently available synthetic pediculicidal agents in the market are cost effective and expensive. Though all of the synthetic pediculicidal agents act efficiently against *Pediculus humanus capitis*, but these products are found to be sometimes neurotoxic also (Vijayalakshmi *et al.*, 2010). Hence non toxic alternative options are needed for treatment of pedoculiasis. Natural source remains less or non toxic and less cost effective. Natural extracts from medicinal plants always act as a rich source for treatment of various diseases and disorders of human system. Recently they are gaining much more importance because of their broad range of pharmacological actions (Muniyandi *et al.*, 2013).

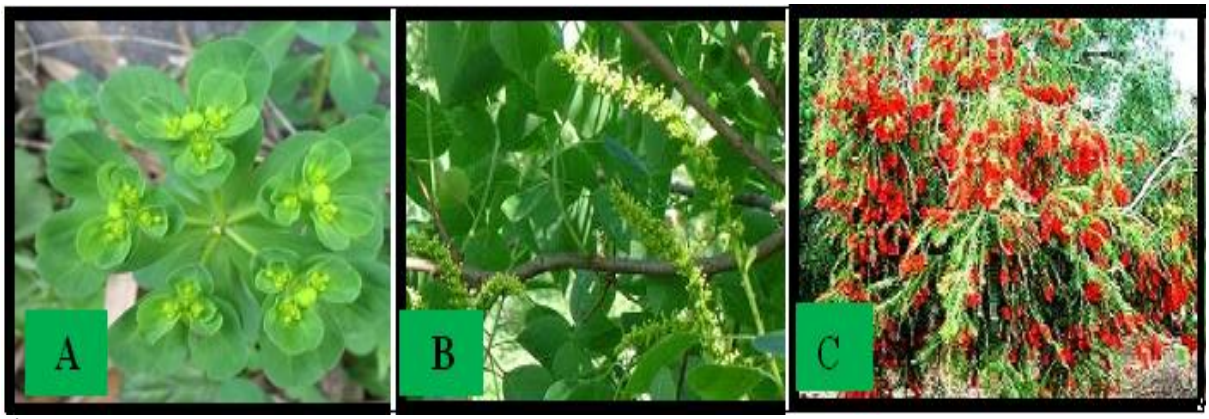

Fig. 1. (A) *Euphorbia helioscopia;* (B) *Sapium sabiferum;* (C) *Callistemon citrinus.*

More than 1000 plants species have been described from many areas which have chemicals components in their seeds, stems, roots, leaves and flowers against insect pests. Among these plants, only few plants have been used to control insects practically on commercial scale in the last few decades. Alkaloids are chemicals which are produced by plants are nitrogenous in nature. These are chemical poison of plants, are heterocyclic in nature and have direct effect on nervous system of animals and cause desolation.

Therefore these alkaloids are named as nerve poisons (Shahid, 2003). Many scientists are now paying their attention to find out such type of plant toxicants, which are environmentally safe and can control these pests in much better way (Lin, 1988; Huang *et al*., 1990; Parihar, 1994; Hutchins, 1997). Study carried out on school children revealed that 20% petroleum ether extract of custard apple seeds killed 95.3% of head lice (Tiangda *et al*., 2000). Another study revealed that Oils from natural plant sources, such as eucalyptus, spearmint, peppermint, cinnamon bark, sage, rosewood, clove bud, and marjoram have exhibited significant pediculocidal activity in filter paper bioassays (Yang, 2003). Synthetic pediculocidal agents, the residue which remains in the head even after rinsing with water gives an enhanced control against lice but also noted for the development of resistance for lice (Meinking and Taplin, 1996). Plant extracts has been noticed for its safe and effective use, and appearance of resistance patterns were minimal due to its different mode of action (Isman, 1996).

In present study experimental plants used against lice were *Euphorbia helioscopia*, *Callistemon citrinus* and *Sapium sabiferum* extracted with two different solvents and targeted specie was *P. humanus capitis*. After treating the filter paper with different concentrations of leaves extract like 100, 200 and 300 ppm. Lice mortality was observed after 6, 12 and 24 hours and results was significantly different ($p<0.05$). In the present study filter paper bioassays was used with three different plants extract i.e. *E.helioscopia, S. sabiferum* and *C.citrinus* organic and inorganic solvent (methanol and water). *P .humanus capitis* were introduced in petri dishes having filter papers soaked in different concentration of the three plant extracts. Different mortality rate was observed in different concentrations of three plants leaves extract in methanol and water solvent at different times. In both solvents mortality rate was higher than control ($p<0.05$). In the leaves extract of *E. helioscopi* and *Sapium sabiferum* is against lice in water solvent percent mortality after 6 hours at 100, 200 and 300ppm was 3.34, 4.34 and 5.67%, 3.34, 4.67 and 6.34%, respectively, which was significantly different from control while in case of *C. citrinus* mortality was 0.00% in all three concentrations. Mortality rate was increased after 12 hours of experiment, for same concentration and observed percent mortality was 5.67, 8.33, 9.34% and 4.67, 6.00, 7.67% for above mentioned both plants in water solvent while remain same in case of *C. citrinus* mortality was 0.00%. Maximum mortality rate was observed after 24 hours and it was 9.67, 12.67, 14.67% and 6.34, 8.67, 12.00% and 0.00, 0.33, and 0.66% for all three plants in water solvent. When the leaves extract of *E. helioscopia*, and *S. sabiferum* were used against *P.humanus capitis* in methanol solvent, percent mortality after 6 hours of experiment at 100, 200 and 300ppm was 5.33, 6.00 and 7.67%, 2.34, 4.00 and 4.34 respectively, which was significantly different from control while and in case of *C. citrinus* no mortality was observed. Mortality rate was increased after 12 hours of experiment for same concentration and observed percent mortality was 8.33, 10.67, 13.67% and 3.33, 5.33, 7.00% for above mentioned both plants in methanol solvent, while again no mortality was observed in case of *C. citrinus*. Maximum mortality rate was observed after 24 hours and it was 13.67, 14.33 and 16.67%, 3.67, 6.00 and 1.93%, 0.34, 0.66 and 1.00% for all three plants in methanol solvent.

Toxicity effect of the 6 extracts against *P.humanus capitis* observed was in following order *E. helioscopia* (methanol) > *E.helioscopia* (water) > *S. sabiferum* (methanol) > *S. sabiferum* (water) > *C. citrinus* (methanol) > *C. citrinus* (water).

The result of this study shows that overall effect of *E.helioscopia* in methanol and water solvent was higher than *S. sabiferum* in both solvents which is further more effective than *C. citrinus* both in water and methanol against *P. humanus capitis* and the extract which was taken in methanol solvent was more effective than extracts in water. This may be due to the presence of some toxic compounds in *E. helioscopia,* which may not be present in *S. sabiferum* and *C. citrinus* or may b in lesser quantity. Here it is concluded that our findings on three plants *E.*

helioscopia, S. sabiferum and *C. citrinus* extracts against *P. humanus capitis* were encouraging and if more attention is given on extraction and purification process of crude plants products than it is possible to increase anti lice potential of these plants. Further studies are needed to characterize and isolate the particular chemical responsible for toxicity in parasites like *P. humanus capitis* and can be put in use on commercial basis after careful experimentations of its safety for its environment and other organisms.

Conclusion

It is concluded that the toxicity effect of the six extracts against *Pediculus humanus capitis* observed was in following order *E. helioscopia* (methanol) > *E. helioscopia* (water) > *S. sabiferum* (methanol) > *S. sabiferum* (water) > *C. citrinus* (methanol) > *C. citrinus* (water).

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