

RESEARCH ARTICLE

IN VITRO PEDICULICIDAL ACTIVITY OF WOOD TAR IN JEDDAH, SAUDI ARABIA

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ABSTRACT

Head Lice, *Pediculus humanus capitis* are the greatest concern global parasites found in all socio-economic classes, affecting mainly the school age children feeding obligatory on human blood of their hosts. Head lice control displays the researcher's challenges to introduce new and safe pediculicides. With increasing the head lice abundance in Jeddah city, the study came to examine some natural products to treat and prevent infestation of head lice, one of them is wood tar solution. The examination procedure included three stages following *in vitro* screening tests, head lice were observed for lack of response to stimuli over three hour period. In preliminary screening wood tar solution showed great activity after 30 to 180 minutes. In secondary screening the LC_{50} and LC_{90} of wood tar solution after 30 minutes were 47% and 62% respectively. In the tertiary stage, the best concentration for wood tar solution was evaluated as ovicidal agents.

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INTRODUCTION

Head lice are obligatory blood feeding ectoparasites on mammals and birds (Durdan, 2001), they feed on blood numerous times at intervals each day (Carter and Davies, 2005), life cycle consist obvious distinguish stages which are eggs, nymphs and adults (Ko and Elston, 2004).

Three effective essential treatment options for head lice are wet combing, topical pediculicides and oral therapy (Elston, 1999). The most effective procedure for pediculosis capitis is using an efficient pediculicide followed by manual nit removal (Burgess, 2006). The active ingredients of pediculicidal treatments currently contain Lindane, Malathion, Pyrethrins and Permethrin (Meinking, 1999).

The antilice activity of aqueous and ethanolic extract of *Dichrostachys cinerea* was studied (Vijayalakshmi, Periyannayagam & Lakshmana, 2010), the ethanolic extract exhibited 98% death in 90 minutes.

Alcoholic, aqueous and hydro alcoholic extracts *Myristica fragrans* pulp was evaluated against head lice at five concentrations, all extracts showed great activity and mortality was recorded at concentrations of 25%, hydro alcoholic extract was found more potent than other extracts (Surendra, Reshma, Nusrath, Dilshad, Sabeer & Babu, 2013).

human adult lice which observed for response lacking to stimuli over three hour period (Shrivastava, Purwal & Jain, 2010). Licatack, which is recently announced as new antilouse agent having grape fruit (*Citrus paradisi*) extracts besides the high quality shampoo constituents, established its activity as pediculicidal and larvicidal product. The whole efficiency was recognized *in vivo* in ten minutes of exposure, when head lice dipped for three minutes *in vitro* within the undiluted shampoo, all were killed (Abdel-Ghaffar, Semmler, Al-Rasheid, Klimpel & Mehlhorn, 2010).

The pediculocidal activity of synthesized silver nanoparticles (AgNPs) using leaf aqueous extract of *Tinospora cordifolia* against head louse were studied (Jayaseelan, Abdul Rahuman, Rajakumar, Kirthi, Santhoshkumar, Marimuthu, Bagavan, Kamaraj, Abdul Zahir and Elango, 2011), the mortality time for synthesized AgNPs was 100% after one hour, where the LC_{50} against head lice was 12.46 mg/L.

The ovicidal effect of chloroform, petroleum ether, methanol, and water extracts of *Pongamia pinnata* leaves was studied (Samuel, Radhamani, Gopinath, Kalusalingam, Vimala & Husain, 2009), all extracts showed concentration ranging from 5% to 20% dependent activity, petroleum ether extract gave greater mortality followed by methanol and chloroform extracts, also petroleum ether extract was a pronounced ovicidal agent. Green tea (*Camellia sinensis*) crude extracts (infusion, decoction and microwave-assisted preparation) were tested as pediculocidal and larvicidal agent by using filter paper experiment and compared with benzyl benzoate as standard drug. All tested extracts presented activity but microwave experiment results were more encouraging gave 100% mortality

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In an effort to evaluate citrus limon juice lethality as pediculicide, juice was examined *in vitro* toxicity against

at 20% and stopped nits hatching at 12th day (Sherwani, Ahmad, Aijaz, Kausar, Sarwar, Mehjabeen & Kazmi, 2013).

Only some products are in markets have repellency affect that keep human hair free from head lice infestation. Combination of *Vitex agnuscastus* seeds extract and paramenthan-3,8-diol (found in *Eucalyptus*) named as Licatack, play synergistically role in protecting human hair from head lice infestation for only seven hours (Semmler, Abdel-Ghaffar, Al-Rasheid, Klimpel & Mehlhorn, 2010). Fumigant and repellent properties of essential oils from 16 native and exotic plants in Argentina, and 21 chemical components against permethrin-resistant head lice were examined. The most effective oil was *Myrcianthes cisplatensis* oil with time of 1.3 minutes (Toloza, Zygadlo, Cueto, Biurrun, Zerba & Picollo, 2006).

Tar can be produced from coal, wood, petroleum and peat, it is a mixture of hydrocarbons and free carbon (Daintith, 2013). Wood tar is viscous black fluid used in pitch, preservatives and medicines (Kaye, 2010). Wood tar was distilled into aqueous fraction, oily fraction and pitch, the oily fraction was distilled into 95 fractions, producing samples for application development and other studies of concentration, separation and purification (Carazza, Rezende, Pasa & Lessa, 1993). Black liquid wood tar looks like Pepsi-Cola, it absorbed through skin that gives it yellow color. It smells like burned paper or very strong burned wood, it resembles form of 90% density tar product of coal, but it completely differs from coal tar (Al-Obeidi, Al-Iraqi & Al-jaf, 2012).

An old study done (Bacot, 1921), wood tar oils were used for destruction males, females and nymphs of head lice by using immersion method for two minutes. Another experiment was conducted by using small drop and large enough to immerse part of insect placed on thorax while it rested on absorbent filter paper. Wood oils were considerably more effective in killing adults but nymphs were more resistant. The aim of work was determine the wood tar activity as pediculicidal agent and to estimate the lethal concentrations that killed 50% and 100% of head lice, as well as to evaluate the wood tar ovicidal property.

MATERIALS AND METHODS

Specimens Collection

Head lice were collected from infested untreated girl students in the elementary schools between ages 7-12 years. Student hair was combed by fine dry lice comb under approval of her schoolteacher or administrator. head lice were collected by putting white paper on chair or table that student sit down, so nearly all head lice would fallen on paper during hair combing. Collected head lice and nits were put in plastic boxes with lids, and transferred immediately to the laboratory after collection.

Wood Tar Solution

The solution was purchased from popular markets in the old area of Jeddah.

Reference Insecticide

Licid shampoo was used as positive control, its active ingredients are bioallethrin and Piperonyl butoxide, and also it consists of oils collection, Olive oil, Chamomile oil, Anise oil and Cactus oil. Licid shampoo was brought from health center located near one of public schools that it distributed free for infested students under the supervision of health unit affiliated school.

Anti-Pediculosis Activity

The experiments protocol was conducted in room temperature of 29 ± 0.5 °C and humidity of $70 \pm 1\%$ in sequences screening.

Primary Screening

Groups of head lice were examined for tested wood tar solution as concentrated material (100%). Head lice were collected from 1- 4 hours as maximum period to avoid starving effect. Filter paper diffusion bioassay was used (Shrivastava, Purwal & Jain, 2010). Ten head lice (adults and nymphs) were placed in filter paper and 1 ml of wood tar was spread over filter paper and head lice. Experiment duration was from 30 minutes to three hours. Head lice were observed every 30, 60, 90 and 180 minutes. Test was done in triplicate and average number of dead lice was considered if there were no symptoms of activity shown. Negative control lice group was spread by distilled water, while positive control were conducted by using Licid shampoo.

Secondary Screening

Gradient dilutions from 0.2 ml - 1ml were prepared from wood tar solution, and same design of preliminary screening for each concentration was used. To remove wood tar trace, 1 ml of distilled water was added in recovery period for one hour. Number of dead lice was counted and time was recorded. Time concentration relationship was documented and estimations of LC_{50} and LC_{90} in different intervals were calculated.

Tertiary Screening

Known age head lice nits were collected from infested students and carried immediately to laboratory. Nit's developmental stage and external markers were used to differentiate between early, medium or late age of nits (Cueto, Zerba & Picollo, 2006), early nits were characterized by absence of external markers, medium nits showed reddish eyes and appendage outlines, and late nits showed black eyes and clearly visible appendages. Nit's activity was microscopically examined under by presence nit's operculum, by noticing embryo heart pulse or limb movement, and by detecting undestroyed shape.

Best concentration of wood tar solution and Licid shampoo were examined for ovicidal activity (Carpinella, Miranda, Almiro'n, Ferrayoli, Almeida & Palacios, 2007). Petri dishes with filter papers were prepared and ten different stages live nits were placed on every filter paper, 1 ml of examined substance was applied just for one time on nits and filter paper and three replicates were conducted. Petri dishes were incubated in Laboratory Incubator at 29 ± 0.5 °C with $70 \pm 1\%$ humidity for 14 days, and 0.1 ml of distilled water at 48 to 72 hours intervals was added to maintain moisture. Incubated nits were checked on

6th day and on 14th day of experiment and hatching were periodically monitored under light microscope. Embryo mortality criteria were nits with closed operculum or nits with opened operculum and embryo inside. Emerged nymphs were counted, and those with incomplete emergence were considered dead.

In repellent activity, three samples of artificial hair tufts were cut and placed into three Petri dishes contained white filter papers (Semmler, Abdel-Ghaffar, Al-Rasheid, Klimpel & Mehlhorn, 2010). One tuft was exposed to the best concentration of wood tar, second exposed to the best concentration of Licid control, third was left without adding any pediculicidal agent, adult head lice were placed aside hair tufts. Test was monitoring for three hours and head lice behavior was noticed visually whether they sheltered away from hair tuft or not.

Statistical Analysis

Median effective concentrations and slope of concentration-percent effect curves were approximated (Leitchfield & Wilcoxon, 1949). Confidence limits for 5% probability were given by method. Effect of different natural products was analyzed by using one way analysis of variance (ANOVA). Least significant differences (L.S.D.) were used as Post Hoc Test to perform pair wise comparisons following significant ANOVA tests.

RESULTS

Mortality rate of *P. humanus capitis* (adult and nymph) was evaluated after treatment for three hours in triplicate examinations. Wood tar solution has strong pediculicidal efficacy (Table 1, Figure 1), after 30 to 180 minutes, no lice movement was observed. The mean death was 9.66 after 30 min and 10 head lice after 60 to 180 min. By using Post Hoc Test, wood tar and Licid shampoo are differ significantly in their effect as pediculicidal agents from distilled water (P=0.000). There is no significant difference between Wood tar solution and Licid shampoo (P=0.836).

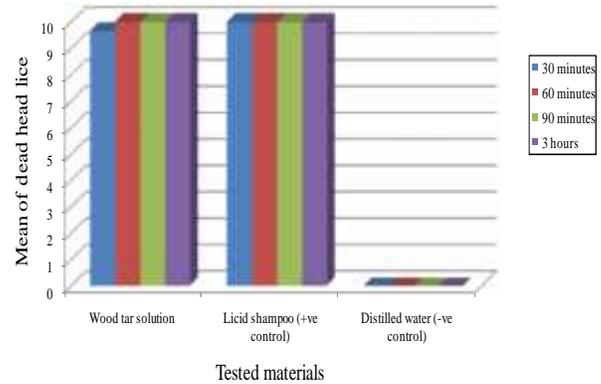


Figure 1 Effect of Wood tar solution and Licid shampoo against adult *P. humanus capitis* after exposing to the tested materials for 30, 60, 90 minutes and 3 hours

Figure 2A: LC₅₀ and LC₉₀ of Wood tar solution with distilled water (equivalent to 1 ml) for *Pediculus humanus capitis* after exposure time of 30 minutes

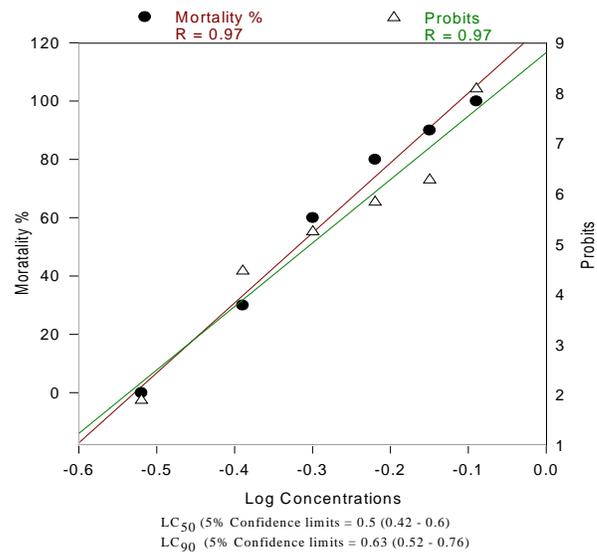


Table 1 Effect of Wood tar solution and Licid shampoo against adult *P. humanus capitis* after exposing to the tested materials for 30, 60, 90 minutes and 3 hours

Wood Tar Solution	No. of head lice used per exp.	Exp. 1		Exp. 2		Exp. 3		Mean	
		Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
30 minutes	10	9	1	10	0	10	0	9.66	0.33
60 minutes	10	10	0	10	0	10	0	10	0
90 minutes	10	10	0	10	0	10	0	10	0
3 hours	10	10	0	10	0	10	0	10	0
Licid shampoo at all time period	10	10	0	10	0	10	0	10	0
Distilled water at all time period	10	0	10	0	10	0	10	0	10

In increasing wood tar concentrations, head lice mortality rate was raised during exposure time (Table 2). Wood tar solution killed all head lice after 30, 60 and 90 minutes in concentrations of 0.8 ml, 0.6 and 0.5 ml respectively. Wood tar solution caused death for 50% of head lice (LC₅₀) and for 90% (LC₉₀) as 0.5 ml and 0.63 ml after 30 minutes (Figure 2A).

After 60 minutes, LC₅₀ was 0.58 ml, LC₉₀ was 0.49 ml (Figure 2B) and they were as 0.37 ml and 0.48 ml after 90 minutes (Figure 2C). Within 3 hours, LC₅₀ was 0.31 ml and LC₉₀ was 0.42 ml (Figure 2D). The ovicidal activity for 14 days was investigated using the best concentrations of wood tar solution, Licid shampoo and distilled water (Table 3, Figure 3).

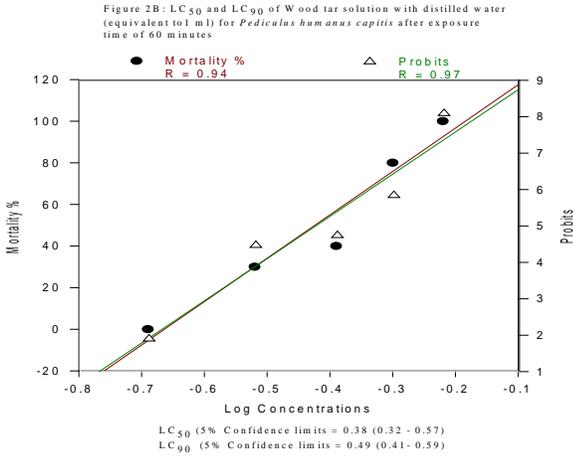


Table 2 Gradient concentrations in 1 ml from Wood tar solution against *Pediculus humanus capitis* after 30, 60, 90 minutes and 3 hours as exposure periods

Material tested	Conc. (%)	No. of head lice used	30 minutes		60 minutes		90 minutes		3 hours	
			Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
Wood Tar	1	10	9	1	10	0	10	0	10	0
	0.9	10	9	1	10	0	10	0	10	0
	0.8	10	10	0	10	0	10	0	10	0
	0.7	10	9	1	10	0	10	0	10	0
	0.6	10	8	2	10	0	10	0	10	0
	0.5	10	6	4	8	2	9	1	10	0
	0.4	10	3	7	4	6	4	6	5	5
	0.3	10	0	10	3	7	3	7	4	6
	0.2	10	0	10	0	10	0	10	1	9
	0	10	0	10	0	10	0	10	0	10
LC Estimation	In 30 min.		In 60 min.		In 90 min.		In 180 min.			
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
	0.5	0.63	0.38	0.49	0.37	0.48	0.31	0.42		

Table 3 Effect of Wood tar solution Garlic, Licid shampoo (+ve control) and distilled water (-ve control) against *P. humanus capitis* nits on two interval times

Experiment days		Materials used					
On the 6 th day	No. of head lice used per exp.	Wood Tar Solution		licid shampoo (+ve control)		Distilled water (-ve control)	
		Hatching	No hatching	Hatching	No hatching	Hatching	No hatching
Exp. 1	10	1	9	0	10	1	9
Exp. 2	10	0	10	1	9	5	5
Exp. 3	10	1	9	2	8	8	2
Mean of dead nits	10	0.66	9.33	1	9	4.66	5.33
On the 14 th day	No. of head lice used per exp.	Wood Tar Solution		licid shampoo (+ve control)		Distilled water (-ve control)	
		Hatching	No hatching	Hatching	No hatching	Hatching	No hatching
Exp. 1	10	1	9	0	10	10	0
Exp. 2	10	0	10	1	9	9	1
Exp. 3	10	1	9	2	8	10	0
Mean of dead nits	10	0.66	9.33	1	9	9.66	0.33

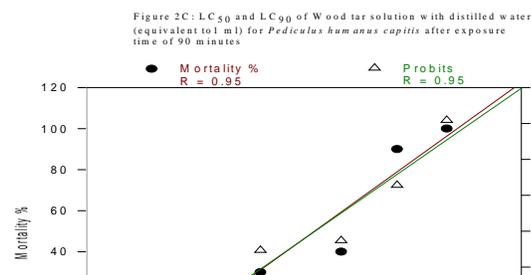


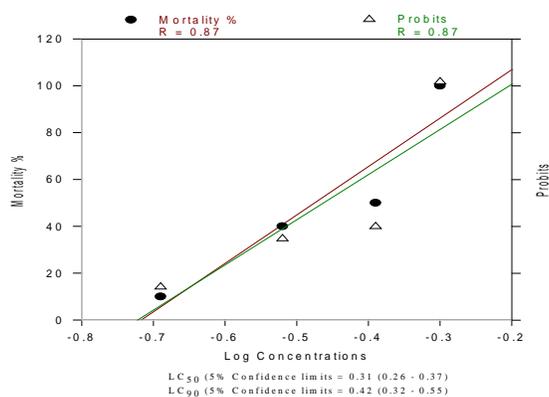
Table 4 Efficacy of Wood tar solution as repellent m... different exposure peri...

	No. of head lice used	After 1 hour				
		+	+/-	-	%	+
Treated Hair with Wood Tar Solution	10	9	1	0	90	9
Normal Untreated Hair	10	0	0	10	0	0

(+): Head lice moved out of hair tuft and were repelled

(+/-): Some of head lice didn't move anymore, the moving ones were repelled from treated hair

Figure 2D: LC₅₀ and LC₉₀ of Wood tar solution with distilled water (equivalent to 1 ml) for *Pediculus humanus capitis* after exposure time of three hours



By using (wood tar solution as ovicidal agent) and on 6th day, average number of non-hatching eggs was 9.33, positive Lcid shampoo showed ovicidal efficacy as 9 eggs did not hatched and 1 egg hatched on 6th day of experiment, using distilled water, head lice nymph were found in Petri dishes as 4.66 eggs and 5.33 eggs did not hatch. In distilled water control hatching average increased from 4.66 on the 6th day.

The antipediculosis effect was differ significantly in its effect as ovicidal agents from distilled water result (P=0.000). There was no significant difference between wood tar solution and Lcid shampoo (P= 0.580) after 14th day of experiment of hatching eggs and non-hatching ones. In first hour of repellent experiment (Table 4, Figure 4), nine head lice went away from hair tuft treated by 0.8 ml of wood tar solution while one louse revolve around hair and then moved away, they never entered hair tuft. In control experiment, head lice were placed close to untreated normal hair tuft and entered it seeking shelter from light.

DISCUSSION

Wood tar result causing lice death as 96.6% which was lower to that mentioned for the ethanol extract of *Dichrostachys cinerea* which showed 98% head lice mortality in 90 minutes (Vijayalakshmi, Periyanyagam & Lakshmana, 2010), but it was better than Citrus limon juice and ½ diluted juice that showed 95±5% and 90±10% respectively (Shrivastava, Purwal & Jain, 2010). Also it was better than that mentioned by Surendra *et al.* (Surendra, Reshma, Nusrath, Dilshad, Sabeer & Babu, 2013), who declared 86.66% of head lice were exposed to death in 18(hours by using 25% alcoholic extract of *Myristica fragrans* fruit pulp. 100% of head lice killed in 18

hours by using 20% *Camellia sinensis* (green tea), these findings are lower than the action 100% for Wood tar solution as pediculicide happened within only 60 minutes (Sherwani, Ahmad, Aijaz, Kausar, Sarwar, Mehjabeen & Kazmi, 2013). Immersion toxicity of median lethal concentrations (LC₅₀ and LC₉₀) were conducted and estimated, which was considered very rare evaluation.

One study was conducted using synthesized silver nanoparticles (AgNPs) using leaf aqueous extract of *Tinospora cordifolia* (Jayaseelan, Abdul Rahuman, Rajakumar, Kirthi, Santhoshkumar, Marimuthu, Bagavan, Kamaraj, Abdul Zahir & Elango, 2011), the LC₅₀ was 12.46 mg/L which was differed from wood tar result that they used nanoparticles and concentrations were measured as mg/L. Wood tar solution as ovicidal agent was lower (93.3%) than that mentioned by Samuel *et al.* (Samuel, Radhamani, Gopinath, Kalusalingam, Vimala & Husain, 2009), who tested petroleum ether extract of *Pongamia pinnata* leaves, and it was better in killing nymph (100%), but it was better than that documented by Sherwani *et al.* who proved that Green tea stopped nits hatching at 12th day while wood tar made the same effect in the 6th day (Sherwani, Ahmad, Aijaz, Kausar, Sarwar, Mehjabeen & Kazmi, 2013). Efficacy of Wood tar solution as repellent material was assessed, and its result was lower than the combination of *Vitex agnuscastus* seeds extract and compound paramenthan-3,8-diol that gave 100% repellency activity at first three hours (Semmler, Abdel-Ghaffar, Al-Rasheid, Klimpel & Mehlhorn, 2010).

In conclusion, Wood tar solution showed highly activity against *p. humanus capitis* and their eggs in low concentration at short exposure periods, and also they gave remarkable effect as repellent agent. Wood tar has advantages properties such as its cheap price, less toxicity, easy obtainable that could be used in herbal formulation as pediculicidal agents.

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