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# Anti head lice activity of *Camellia sinensis* (Green tea) aqueous decoction, infusion and microwave assisted crude extracts

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## ABSTRACT

Pediculosis caused by *Pediculus humanus capitis* (head lice) is one of the serious health problems worldwide. In the current study, *Camellia sinensis* crude extracts (infusion, decoction and microwave assisted preparation) were exploited for anti-lice property by using filter paper assay. Four different concentrations such as 5, 10, 15 and 20% of the tests and standard showed dose dependent activity when compared with standard drug benzyl benzoate. The results highlighted that although; all extracts possessed activity yet the microwave preparation results were more promising as showed 100% mortality of adult lice at 20% and even did show any sign of emergence of nits even at 12<sup>th</sup> day. Results conclude that if bioactive is searched for from microwave assisted extract that could be incorporated in shampoo products.

**Keywords:** *Camellia sinensis*, Benzyl Benzoate, *Pediculus humanus capitis*, Anti-Head Lice

## 1. Introduction

*Pediculus humanus capitis* (head louse) is an ectoparasite firmly attached to the scalp of hair of humans<sup>[1]</sup>. Many studies have reported that infestations are prevalent in all age groups however; especially common among school children between the ages of 5-11 years in developed as well in developing nations<sup>[2]</sup>. Infestation rates have been reported higher among girls than boys<sup>[3]</sup>. The transmission of head lice is believed to occur primarily via direct head to head contact as well as by means of personal belongings of infested individual articles like combs, brushes, hats etc<sup>[4]</sup>. *Camellia sinensis* L. commonly called tea is largely consumed since time of immemorial and has immense scientific significance for its numerous therapeutic properties mainly because of polyphenolic compounds<sup>[5]</sup>. Only young leaves are mostly used for drinks and extracts, however, many parts of this plant are being explored for benefit of mankind<sup>[6]</sup>. Depending on tea manufacturing methods, tea is divided mainly into: green and the black one<sup>[7]</sup>. Green tea is generally safe, non-toxic and having no side effects after use<sup>[8]</sup>. Attempt of botanical insecticides have been in practice since long in some form or the other for controlling insect pests<sup>[9]</sup>. The current study is undertaken for to evaluate the potential of various crude *Camellia sinensis* (green tea) extracts as anti- head lice agent.

## 2. Material and methods

### 2.1 Purchase of Green tea

The Green tea was purchased from a common shop in whole sale price in Karachi-Pakistan and was brought in the research laboratory of Federal Urdu University of Arts, Science and Technology (FUUAST)-Karachi-Pakistan for making extracts and conducting a bioassay as indicated in Fig 1.

### 2.2 Preparation of Infusion

The aqueous infusion was prepared in 5% concentration i.e. by taking 5gm of *Camellia sinensis* (Green tea) in 100 ml distilled water and left for about 48 hours with occasional shaking at room temperature and later filtered by strainer to procure clear infusion and then also pass through 0.22um filter and store the infusion into small eppendorf vials in freezer for further work<sup>[10]</sup>.

### 2.3 Preparation of Decoction

The aqueous decoction was prepared by boiling in 5% concentration i.e. 5 gm *Camellia sinensis* (Green tea) in 100 ml distilled water in a flask for 15-20 minutes. The flask was removed from heat and allowed to cool. The content of flask was filtered to procure clear decoction and then also pass via 0.22 µm filter and store the infusion into small eppendorf vials in freezer for further work [11].

### 2.4 Microwave Extract preparation

The tea was prepared in 5% concentration i.e. 5 gm were added into flask separately and then 100 ml of distilled water was added in all of them. Microwave assisted extraction carried out by using 245 W [12]. The extraction was completed for 15 min constantly without any disturbance in order to avoid mishap or accident [13].

### 2.5 Concentration of Test and standard drug

*Camellia sinensis* (Green Tea) infusion, decoction and microwave based extracts as well as standard drug were prepared in four different concentrations i.e. 5, 10, 15 and 20% for the sake of bioassay.

### 2.6 Collection of head lice

*P. humanus capitis* were collected from school going children between the age group of 8-12 from some school children located in the city Karachi-Pakistan after getting permission of the head master/head mistress by combing using a clean, sharp teeth white comb. After careful collection of lice, nymphs and nits, they were kept in sterile small polypropylene (disposable) petri plates containing human hair strands. The lid was loosely sealed by parafilm wax to permit ventilation and immediately transported to the research laboratory-Department of Microbiology, Federal Urdu University Of Arts, science and Technology-Karachi-Pakistan. The in-vitro tests for head lice and ovicidal activities were started within 1 hrs after collection. The inclusion criterion was including subjects who had not been treated with any anti-lice Shampoo or products for the preceding 3 months. After careful selection under a dissecting microscope, the adults and nymphs were identified and separated.

### 2.7 Anti-lice activity

All three aqueous crude preparations of green tea were subjected for anti-lice activity by filter paper diffusion technique. All four different concentrations (5, 10, 15 and 20%) were tested. After careful selection under a dissecting microscope, the adults and nymphs were identified and separated. All the test organisms in a ratio of 3.6/1.4 (adult/nymph) were divided into 4 alphabetical groups A, B, C, D, E (5 lice each) and were placed on a filter paper at the bottom of petri dish and kept open. A 0.5 ml of each test samples was poured on the test organisms and allowed to spread as a thin layer of 4 cm<sup>2</sup>. Group A was treated with 0.5 ml distilled water and served as control. Group B, C and D (test groups) received 0.5 ml of various concentrations of aqueous, petroleum ether and methanol extracts respectively. Group E was treated with 0.5 ml of 5, 10, 15 and 20% of benzyl benzoate 25% (w/v). All the Petri dishes were set aside for 1 hr in a dark and humid hood [14]. At the end of 1 hr, the dishes were taken out and applied 0.5 ml of distilled water and further placed in the chamber under the condition mentioned above. After 18 hr, the dishes were observed under microscope for any

possible movement of lice. The criteria used for survival of lice were extremely strict and clear if any minor signs of life, such as movements of antennae or minimal leg movements were noted (with or without stimulation by a forceps), the lice were categorized as alive. The lice were judged as dead if there were no vital signs at all.

### 2.8 Ovicidal activity

The ovicidal activity was tested by following the method with slight modifications i.e. by placing almost 5 brownish oval eggs on the filter paper (Whatman No. 1; 6 cm diameter) placed in the bottom of each petri dish. Then, 0.5 ml of each test solution and control were applied on the nits. All the dishes were then incubated in a dark box for 16 days. To maintain the moisture, 0.1 ml of distilled water was added at 24 hrs interval. Hatching of eggs were monitored under a microscope and the percentage of emergence, i.e. partially hatched nits were observed and the findings were recorded [14].

**Table 1:** Colour of *Camellia sinensis* (green tea) crude extracts by various methods

S:No	<i>Camellia sinensis</i> (Green tea) extract preparations	Colour
1	Infusion extract	Light brown
2	Decoction extract	Golden brown
3	Microwave based extract	Dark brown

**Table 2:** Percentage yield of *Camellia sinensis* (Green tea) various extracts

% Yield (Infusion) (w/w)	% Yield (Decoction) (w/w)	% Yield (Microwave method) (w/w)
16.7	19.2	23.6

**Table 3:** Activity of various crude extracts of *Camellia sinensis* (Green Tea) against *Pediculus humanus capitis* adults and nymphs

Test compounds	Concentrations (%)	Average mortality (%)
Autoclaved distilled water	-	
Infusion	5	20.6
	10	41.8
	15	58.9
	20	69.7
Decoction	5	46.3
	10	66.4
	15	77.2
	20	89.5
Microwave based extract	5	53.8
	10	77.9
	15	97.3
	20	100
Standard drug	5	70.7
	10	86.5
	15	98.7
	20	100

**Table 4:** Activity of various crude extracts of *Camellia sinensis* (Green Tea) against *Pediculus humanus capitis nits*

Test compounds	Concentrations (%)	Emergence (%)	
		6 <sup>th</sup> day	12 <sup>th</sup> day
Autoclaved distilled water	-	86.4	95.6
Infusion	5	74.3	56.2
	10	46.1	33.3
	15	21.1	15.4
	20	16.8	9.6
Decoction	5	77.1	70.1
	10	65.4	44.2
	15	54.2	23.7
	20	46.5	14.7
Microwave based extract	5	27.9	12.6
	10	12.2	9.6
	15	6.6	0
	20	0	0
Standard drug	5	5.5	0
	10	0	0
	15	0	0
	20	0	0

**Fig 1:** View of commercially available green tea purchased from the local retail market- Karachi-Pakistan for performing anti-headlice activity**Fig 2:** Collection of head lice from a School boy by combing white sharp teeth fine comb on the scalp of the head in Karachi-Pakistan.

### 3. Results and Discussion

Pediculosis is distributed all over the world affecting seriously various ethnic groups regardless of sex and socioeconomic

conditions<sup>[15]</sup>. The therapeutics derived from plants have been used against biting arthropods since long and such practice was noted in almost in civilizations<sup>[16]</sup>. In order to get rid of human head lice synthetic pediculocides are being used<sup>[1]</sup> but their insensible and repeated use results in development of resistance<sup>[17]</sup>. In the present study, for performing anti lice activity, head lice were collected from school going boy as they usually catch from other mates as mentioned in Fig1.

**Fig 3:** Adult louse on the white sharp teeth comb after collection from a school boy in Karachi-Pakistan.

Moreover, better understanding and conclusion, intentionally, adult lice were included with a certain visible distinct size, the rest were killed manually and not included in research study as shown in Fig 2. The current study indicated this fact that all three way of *Camellia sinensis* (Green tea) extract preparations i.e. decoction, infusion and microwave preparations showed anti head lice and ovicidal activity. All three decoction, infusion and microwave preparations gave different colours like golden brown, light brown and dark brown respectively as mentioned in Table 1. Moreover, variable % yield was obtained as infusion extract gave 16.7% (w/w), decoction 19.2% (w/w) and highest yield was procured in microwave assisted procedure 23.6% (w/w) as indicated in Table 2. However; the effectiveness level was found differently in all three cases. All four different concentrations (5%, 10%, 15% and 20%) of test drug and standard were tested. Benzyl benzoate 25% (w/v) was used as a standard and a known effective adulticidal and ovicidal agents. The results pointed out the microwave assisted preparation showed promising activity in terms of inhibition and further emergence as compared to decoction (boiled) that showed moderate and infusion that have very least activity. This probably due to this fact as thought that different treatment may release different nature of biologically active substances. The microwave and decoction preparations based at actually upon heat treatment that destroy the leaf cells resulting in the release of all possible natural phyto-constituents that could play very definite and effective role in the biological activity. A very popular example is neem having a very active

ingredient in pesticidal skin cream as well as shampoo<sup>[18]</sup>. In our study, four different concentrations of the test and the standard in an ascending order such as 5%, 10%, 15% and 20% showed dose dependent activity when compared with standard drug benzyl benzoate. The results highlighted that although; all extracts possessed activity yet the microwave preparation results were more strikingly significant as showed 100% mortality of adult lice at 20% and 0% emergence of nits was observed at 12<sup>th</sup> day. Moreover, fairly good activity was also found in case of decoction, i.e. 89.5% killing of adult lice at 20% concentration of test drug tested and 14.7% emergence was noted at 12<sup>th</sup> day. On the contrary, infusion results was low as showed 69.7% of killing of adult lice and exhibited 9.6% emergence of nits at 12<sup>th</sup> day as indicated in Table 3 and 4. *Camellia sinensis* (green tea) possessed 700 important chemicals and vitamins thus having very rich composition<sup>[19]</sup>. Studies pointed out that green tea possessed important medicinal pharmacological active substances<sup>[20]</sup>. Some of the significant phytoconstituents that have profound beneficial effect on human health are polyphenols, tannins and catechins<sup>[21]</sup>. A same of researchers have conducted enough studies in exploring anti head lice potential of oils extracted from natural sources, such as eucalyptus<sup>[22]</sup>, marjoram, spearmint<sup>[23]</sup>, peppermint, sage<sup>[24]</sup>, clove bud, and cinnamon bark<sup>[25]</sup> have found wonderful pediculocidal activity in filter paper bioassays.

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