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In Vitro Screening of Anti-lice Activity of *Pongamia pinnata* Leaves

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Abstract: Growing patterns of pediculocidal drug resistance towards head louse laid the foundation for research in exploring novel anti-lice agents from medicinal plants. In the present study, various extracts of *Pongamia pinnata* leaves were tested against the head louse *Pediculus humanus capitis*. A filter paper diffusion method was conducted for determining the potential pediculocidal and ovicidal activity of chloroform, petroleum ether, methanol, and water extracts of *P. pinnata* leaves. The findings revealed that petroleum ether extracts possess excellent anti-lice activity with values ranging between 50.3% and 100% where as chloroform and methanol extracts showed moderate pediculocidal effects. The chloroform and methanol extracts were also successful in inhibiting nymph emergence and the petroleum ether extract was the most effective with a complete inhibition of emergence. Water extract was devoid of both pediculocidal and ovicidal activities. All the results were well comparable with benzoyl benzoate (25% w/v). These results showed the prospect of using *P. pinnata* leave extracts against *P. humanus capitis* in difficult situations of emergence of resistance to synthetic anti-lice agents.

Key words: *Pongamia pinnata*, leaves, anti-lice activity, filter paper bioassay, head louse

INTRODUCTION

Pediculus humanus capitis, otherwise called as the human head louse, infestation is a major concern in public health-associated problem. Head lice are ectoparasites and its infestation due to unhygienic conditions has negatively affected the society for decades, back to the earliest *Homo sapiens*. The condition is distributed around the world invading various ethnic groups with no restrictions of sex and socioeconomic status [1]. In Malaysia, people buy costly products in combating head lice and the money they plough into annually seems greater when they realize that the products they used were apparently ineffective [2]. This lack of efficacy is due to the emergence of resistance by the head louse to synthetic compounds and researchers were aimed on the search of new substitutes to synthetic ingredients, such as phytoconstituents obtained from plant sources [3,4].

Pongamia pinnata Linnaeus (Fabacae) is a medium-sized glabrous tree distributed along the coasts and river banks in India and Myanmar. Native to the Asian subcontinent, this species has been introduced to humid tropical lowlands in the Philippines, Malaysia, Australia, Seychelles, United States, and Indonesia [5]. Pongamol and karanjin isolated from fruits of *P. pinnata* were reported to have antihyperglycemic activity [6]. The oil obtained from this plant is used for the treatment of rheumatism, scabies as well as human and animal skin diseases in folk medicine [7]. Juices of leaves are used in curing cold, coughs, diarrhea, dyspepsia, flatulence, and gonorrhea. Dried leaves are used as an insect repellent in stored grains, and also used as a pesticide [8]. The ethanomedical claim by local tribes in Malayasia (Orang Asli) uncovered that *P. pinnata* is a potential source for controlling pediculosis. On these grounds of traditional claim, we have decided to screen various extracts, such as chloroform, petroleum ether, methanol, and water of *P. pinnata* leaves on adults, nymphs, and nits for its potential anti-lice and ovicidal activities.

MATERIALS AND METHODS

Plant material

Fresh leaves of *P. pinnata* were collected from Taman Alam Jaya, Selangor, Malaysia in April 2008. The samples were iden-

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tified and authenticated by Dr. Sani Miran, botanist, National University Malaysia, Selangor, Malaysia. A voucher specimen (MUCHH/PH12/PP03) was deposited at the herbarium, School of Pharmacy, Masterskill University College of Health Sciences, Cheras, Selangor, Malaysia. The leaves were washed under tap water to remove debris and dried under shade for 10 days. The dried leaves were size-reduced to coarse powder in a mill.

Extraction

The coarse powdered leaves of *P. pinnata* (1,000 g) were extracted successively with petroleum ether, chloroform, methanol, and water by Soxhlet extraction technique [9]. All the extracts were concentrated using rotary vaccum evaporator and kept in a dessicator until further studies. The color, consistency, and percentage yield were observed.

Collection of head lice

Adults, nymphs, and nits of *P. humanus capitis* were collected from children between the age group of 8-12 by combing through sections of the scalp using a clean comb. After combing, the lice were carefully removed from the teeth of the comb into plastic boxes. All the subjects had not been treated with any anti-lice products for the preceding 3 months.

Anti-lice activity

Petroleum ether, chloroform, methanol, and water extracts of *P. pinnata* were tested for pediculocidal activity by filter paper diffusion method [10]. All the extracts were dissolved in distilled water to obtain 3 different concentrations (5%, 10%, and 20%). 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 16 groups (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^2. Group 1 was treated with 0.5 ml distilled water and served as control. Group 2 to group 13 received 0.5 ml of various concentrations of aqueous, petroleum ether, chloroform, and methanol extracts respectively. Group 14 to group 16 were treated with 0.5 ml of 5%, 10%, and 20% of benzyl benzoate 25% (w/v) (RidPed). All the Petri dishes were set aside for 1 hr in a dark chamber at 26 ± 0.5℃ and 70 ± 1% humidity [11]. 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 a dissecting microscope for any possible movement of lice and absence of any movement were considered dead [12]. All the treatment was triplicate.

Ovicidal effects

The ovicidal activity was tested by placing 5 brownish oval eggs with an unbroken operculum on the filter paper (Whatmann 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 chamber at 26 ± 0.5℃ for 14 days. To maintain the moisture, 0.1 ml of distilled water was added at 48 hr interval. Hatching of eggs was monitored under a microscope and the percentage of emergence, i.e., partially hatched nits, was observed, and the findings were recorded [11]. Each treatment was replicated 3 times.

RESULTS

The color, consistency, and percentage yield of petroleum ether, chloroform, methanol, and water extracts of *P. pinnata* were recorded in Table 1. All the extracts displayed concentration (5%, 10%, and 20%) dependent activity among which petroleum ether extract showed higher mortality followed by methanol and chloroform extracts, respectively and was well comparable with the standard. Water extract in various concentrations showed minimal anti-lice activity (Table 2).

The findings of ovicidal activity of *P. pinnata* extracts were tabulated in Table 3. Petroleum ether extract showed pronounced ovicidal activity by delaying the emergence of nymph on the 6th day and 14th day and completely inhibited the emergence similar to that of the standard. Methanol and chloroform extracts moderately delayed emergence of nymph, whereas water extract was devoid of ovicidal activity.

DISCUSSION

The use of *P. pinnata* extracts for controlling lice infestations has been authenticated from the excellent results obtained after

Table 1. The color, consistency, and percentage yield of extracts

Extracts	Color	Consistency	% yield
Petroleum ether	Pale greenish	Sticky mass	6.0
Chloroform	Greenish	Sticky mass	9.0
Methanol	Greenish brown	Semi solid	18.0
Water	Dark green	Semi solid	23.0

Table 2. Effects of *Pongamia pinnata* leaf extracts against *Pediculus humanus capitis* adults and nymphs

Test sample	Concentration (%)	Average mortality[a] (%)
Distilled water (0.5 ml)	-	9.6
Petroleum ether extract (0.5 ml)	5	50.3
	10	88.7
	20	100
Chloroform extract (0.5 ml)	5	31.4
	10	43.1
	20	76.9
Methanol extract (0.5 ml)	5	17.3
	10	32.6
	20	82.9
Water extract (0.5 ml)	5	10.3
	10	10.7
	20	18.7
Benzoyl benzoate 25% w/v (0.5 ml)	5	60.7
	10	100
	20	100

[a]n = 3.

Table 3. Effects of *Pongamia pinnata* leaf extracts against *Pediculus humanus capitis* nits

Test sample	Concentration (%)	Emergence[a] (%)	
		Day 6	Day 14
Distilled water (0.5 ml)	-	81.3	91.6
Petroleum ether extract (0.5 ml)	5	20.8	6.9
	10	0	0
	20	0	0
Chloroform extract (0.5ml)	5	56.2	41.3
	10	52.1	28.7
	20	21.6	11.4
Methanol extract (0.5 ml)	5	61.8	51.9
	10	47.1	24.4
	20	38.9	9.3
Water extract (0.5 ml)	5	62.3	45.5
	10	29.4	14.8
	20	18.6	10.4
Benzoyl benzoate (25% w/v) (0.5 ml)	5	6.1	0
	10	0	0
	20	0	0

[a]n = 3.

screening various extracts for potential anti-lice and ovicidal activity. Oils from natural sources, such as eucalyptus, marjoram, spearmint, peppermint, sage, rosewood, clove bud, and cinnamon bark have exhibited significant pediculocidal activity in filter paper bioassays [13-16]. Another study carried out on school children revealed that 20% petroleum ether extract of custard apple seeds killed 95.3% of head lice [17]. Previous literatures on phytochemistry of *P. pinnata* seeds reported the presence of sterol and its derivatives together with saturated and unsaturated fatty acids (monoenoic, dienoic, and trienoic acids) establishing its lipophilic nature [13].

The findings of this study showed excellent anti-lice and ovicidal activities of petroleum ether extract of *P. pinnata* which may be due to the presence of these sterol derivatives responsible for the enhanced penetration and bioavailability of oil components into the body of louse. Penetration of extracts into the alimentary tract of lice could be ignored since all the extracts was applied on lice placed on the filter paper which also subsequently avoided immense dissemination of active constituents into the cuticle when the compound is directly applied to the insect skin [18]. 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. For 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 [19,20]. Natural extracts from medicinal plants has been noticed for its safe and effective use, and appearance of resistance patterns were minimal due to its different mode of action [21,22] which greatly supports the safe use of *P. pinnata* extracts as a potent anti-lice agent.

Eradication of lice would be complete if the products used for pediculocidal activity also delays nymph emergence and potentially kill the nymph. Extracts of *P. pinnata* succeeded in delaying the emergence of nymphs, and its oily nature may help to detach nits from the hair before hatching. Among all the extracts, petroleum ether exhibited the maximum pediculocidal effects and completely inhibited nymph emergence at 2 different concentrations (10% and 20%). Hence, the results obtained from this research present a promising scenario for using *P. pinnata* leaf extract as an effective alternative for treating human head lice.

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