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Research Article

Phytochemical Study And Evaluation of Pediculicidal Activity of Herbal Plant Extracts of *Pongamia pinnata, Annona squamosa, Tephrosis purpurea, Cassia obtusifolia and Melaleuca alternifolia*

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Abstract

Background: Head louse (*Pediculus humanus capitis*) infestation is a significant public health issue in humans. Lice are becoming increasingly resistant to pediculicidal drugs; anti-lice chemicals have lost their efficacy, and hence substitutes for these drugs from medicinal plants have been proposed to treat lice infections. Objective: In this study report plant extracts from *Annona squamosa* (custard apple) (Family: Annonaceae) seed, *Cassia obtusifolia* (senna, sonamukhi) (Family: Leguminosae) seeds, *Melaleuca alternifolia* (tea tree) (Family: Myrtaceae) leaves, *Pongamia pinnata* (karanj) (Family: Fabaceae) leaves, and *Tephrosia purpurea* (unhali) (Linn.) (Family: Fabaceae) seeds were tested against the head louse. Method: Active constituents from leaves and seeds of medicinal plants were extracted using petroleum ether. The lice mortality time was assessed with the diffusion bioassay method using filter paper. Extracts of different concentrations (5% w/v, 10%w/v, 20% w/v, 30% w/v, 40% w/v, and 50%w/v) were analyzed for mortality. Result: Preliminary phytochemical screening showed the occurrence of bioactive compounds, especially carbohydrates, tannins, proteins, alkaloids, saponins, flavonoids and steroids in all extracts. The results were compared with chemical pediculicide (positive control; 1% w/w licel). Conclusion: The finding revealed plant extracts possess anti-lice activity at a concentration of 50% v/v.

Keywords: Phytochemical Screening, Plant Extracts, Anti-lice Activity, Pediculosis, Bioactive Compounds

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Introduction

Pediculosis (louse infestation) is an infection of the human scalp brought on by *Pediculus humanus capitis* (Phthiraptera: Pediculidae). Its invasion is worldwide each year and has been observed in most of the countries. The parasitic lice on human beings are *Pediculus humanus capitis, Pediculus humanus*, and *Pthirus pubis*, which inhabit the head, body, and pubis, respectively. Among these head louse is a small, wingless obligate ectoparasitic insect (1). The egg, nymph and adult stages of lice require human host body regions. Their transmission occurs directly through skin-to-skin or passive vectors, and symptoms may be observed after 21 to 28 days.

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It causes public health problems, especially in poor sanitary conditions (1). However, it is believed that 300 million cases of pediculosis occur annually and affect people all over the world, particularly young children. The principal signs of lice infection include skin inflammation, allergies, lack of sleep, intense itching of the scalp etc. Additionally, head lice infestation can result in secondary bacterial infections such as Acinetobacter baumannii, Bartonella quintana and Borrelia recurrentis. The lice infection can be diagnosed with the presence of dandruff in hair, seborrhea, eczema, folliculitis, surface fungal infection, scabies, etc. Normally, the head lice transmission may be direct, from one person to another or indirect, through clothing, common use of combs, bedding, blankets etc. Human head lice infestation has increased worldwide, particularly in age groups of 5-11 years among schoolchildren (2, 3). The manifold relevant pediculosis treatments comprise pyrethroids, Malathion, lindane, benzyl alcohol, topical and oral ivermectin, and spinosad which are the first-line treatment (4). Though the chemical control of head lice mainly depends on insecticides such as organo-chlorine (lindane), pyrethroid (pyrethrin), organo-phosphate (Malathion), carbamate (carbaryl), unfortunately, many of these failed to obtain lice

control. It has been reported that head lice have increasing resistance against chemical insecticides in many countries such as the USA, Argentina, England, and Australia (5, 6). Head Lice have developed resistance to permethrin as recently reported. After its use, it has residue on hair and can last for up to three weeks (7). An organophosphate cholinesterase inhibitor, Malathion 0.5% paralyses the respiratory system of arthropod invertebrates. It is found to be safe but has a nasty odor. In such cases, a treatment period of 8-12 hours is recommended. An organochloride like 1% Lindane kills lice through respiratory paralysis. It is engrossed slowly into the blood, and metabolized. Its frequent use should be avoided. It has been withdrawn from the market in California as its capability for neurotoxicity (8). Topical ivermectin 0.5% lotion causes hyperpolarisation and paralysis via raising chloride levels in muscle cells (9).

Although some synthetic pediculicidal chemicals are highly effective, costly and neurotoxic against P. h. capitis, hence it became necessary to find safe and non-toxic alternative agents for the treatment of head lice. Traditional medicine derived from nature has been used since prehistoric times because they are affordable, readily available, and less poisonous as compared to synthetic products. Besides this, natural products are potentially degradable and compatible. Thus, alternative anti-lice remedies containing active ingredients derived from medicinal plants are required (10). Many herbal extracts are considered safe alternatives for the management of head lice due to their traditional acceptability, availability, and easily biodegradable (11) and fewer side effects than shampoo. The curative plants selected for the study of anti-lice activity purpose were *Annona squamosa* (12), *Cassia obtusifolia* (Sickle pod or Coffee Weed), *Melaleuca alternifolia*, *Pongamia pinnata* (10), and *Tephrosia purpurea*. These plants have therapeutic properties as listed in Table 1. Developing resistance day by day to pediculicidal drugs by head louse motivated us to undertake this study. Our attempt at the study was to examine the potential of these medicinal plant extracts against head lice and compare it with licel. Here in this study, an attempt was made to check the effect of *Annona squamosa* (custard apple) (Family: Annonaceae), *Cassia obtusifolia* (senna, sonamukhi) (family – Fabaceae/ Leguminosae), *Melaleuca alternifolia* (tea tree) (family-Myrtaceae), *Pongamia pinnata* (karanj) (Family: Fabaceae), and *Tephrosia purpurea* (unhali) (Family: Fabaceae) against lice. More research is needed to understand its potential and safety, especially for larger-scale treatments.

Table 1: List of five species from herbs, part used and therapeutic property in this study

Scientific Name	Part used	Therapeutic property
Pongamia pinnata	Leaves	Anti-cacner, skin diseases, ulcers, piles, stomach ache, anti-inflammatory, antidiabetic, wound healing, bronchitis, whooping cough (1,21)
Melaleuca alternifolia	Leaves	Lice, acne, athlete's foot, nail, fungus, skin healing (10)
Annona squamosa	Seeds	To treat urinary tract infection, dysentery, antioxidant, cytotoxic, antimalarial, antidiabetic (12,13)
Tephrosia purpurea	Seeds	Anthelmintic, nutritive, Antipyretic, rheumatism, dyspepsia, Antidiarrheal
Cassia obtusifolia	Seeds	Diuretic, laxative, tonic, purgative, treating headache, dizziness, constipation (10)

Materials and methods

Plant material

Collection and identification

The leaves of *Pongamia pinnata, Melaleuca alternifolia* and seeds of *Annona squamosa*, *Tephrosia purpurea*, *Cassia obtusifolia* were collected from rural area Mulshi, Pune and washed with water, shade-dried and were pulverized with a mechanical grinder (13-14). All herbs identification and authentications was made by Botanical Survey of India, Pune.

Collection of head lice

The adult lice of the genus P. h. capititis which were not previously treated with any chemical or herbal insecticides for at least 1 month collected from children age group of 5-16 years (11). The consent of the children's guardians was taken. The lice were collected by combing through sections of the scalp with the help of a clean fine-toothed comb. Those were then stored in plastic containers of size 6"x3"(6). All collected live specimens of head lice were sent to Laboratory of JSPM's Charak College of Pharmacy and Research, Wagholi, Pune. The head lice were treated in-vitro with previously prepared extracts within one hour of their collection. The head louse specimens were kept in Laboratory for further reference.

Figure 1: Different parts of plants used for study

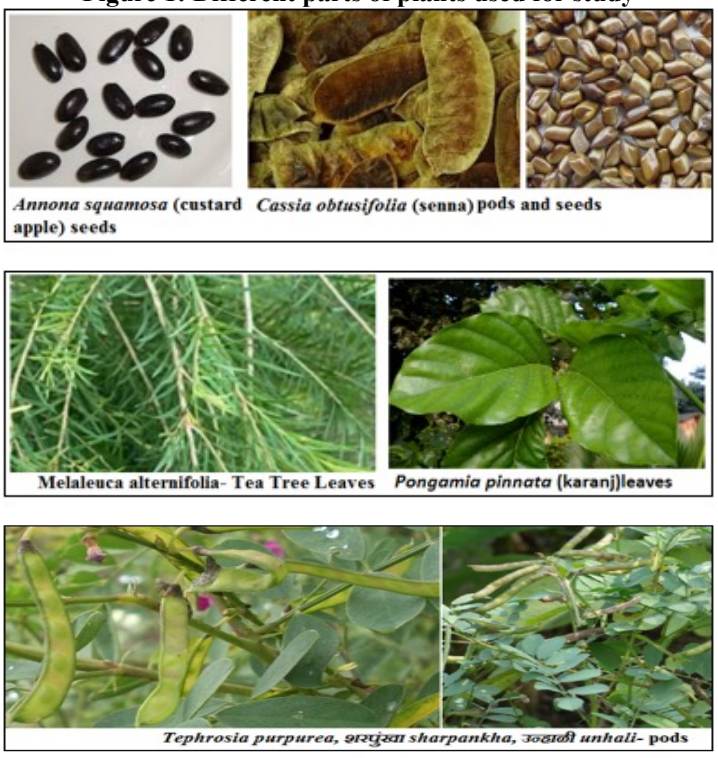


Figure 2: Collection of head lice

Methods

Extraction and isolation

The dried powdered leaves & seeds of given medicinal plants were extracted successively with petroleum ether by Soxhlet extraction technique (15,16). All the extracts were concentrated using rotary vacuum evaporator and store in refrigerator until further studies. The color, consistency and percentage yield of all extracts were observed.

Preliminary phytochemical analysis

The prepared herbal extracts were subjected to qualitative chemical analysis for identification of various phytoconstituents like carbohydrates, proteins, saponins, glycosides, tannins, aminoacids and terpenoids by using different method (17, 18).

Bioassay

Herbal extract and its mixture were tested for licicidal activity by filter paper diffusion method. Individual plant extracts dilutions were prepared in concentration 5%, 10 %, 20 %, 30 %, 40% and 50 % w/v using distilled water. After a careful selection of head lice under a projection microscope, filter papers discs (Whatmann No 1) were placed at the bottom of Petri dishes. Total 10 head lice (adults) were placed in each petri dish and then 1 ml of each test sample of each concentration was poured on the test organisms and allowed to spread as a thin layer of 4 cm^2. Negative control, head lice were placed directly on the filter paper with distilled water (without any treatment). The head lice as dead or alive were observed under microscope. Mortality time was recorded at different concentrations. 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. The activity study was carried out in triplicate (7).

Results

Lice infestations is distributed worldwide whereas synthetic pediculicides are expensive. However, all of the synthetic pediculicidal agents are highly effective against P. humanus capitis, some of them are neurotoxic. Hence safe and non toxic alternative options are needed for treatment of head lice. Natural products have been used in traditional medicine for thousands of years because they are inexpensive, readily available, less toxic when compare with synthetic products, potentially degradable and compatible due to their natural origin. We made an attempt to check the effect of different plant extract for antilice activity. The Phytochemical evaluation of petroleum ether extract of all plants shown presence of various phytoconstituents like carbohydrates, proteins, saponins, glycosides, tannins, aminoacids and terpenoids (table 2).

Table 2: Phytochemical analysis of plant extract

Sr. No	Test for plant constituent	*Tephrosia Purpurea*	*Annona Squamosa*	*Cassia Obtusifolia*	*Pongamia Pinnata*	*Melaleuca alternifolia*
1	Carbohydrates	+	-	-	+	-
2	Proteins	-	+	+	-	-
3	Alkaloids	+	-	+	+	-
4	Flavonoids	+	+	+	+	+
5	Tannins	+	+	+	+	-
6	Steroids	+	+	+	+	-
+ = Presence - = Absence						

The herbal extract of seeds of Annona squamosa (custard apple), Cassia obtusifolia (senna, sonamukhi), Tephrosia purpurea (unhali) and leaves of Melaleuca alternifolia (tea tree), Pongamia pinnata (karanj), (Figure 1) was prepared at concentrations of 5, 10, 20, 30, 40 and 50 % w/v for lice treatment. The results shown that as concentration of medicinal plant extract increases, 100 % mortality of lice achieved in short time (table 3).

Figure 3: (A) Head louse under a microscope (B) Licicidal activity of plant extracts

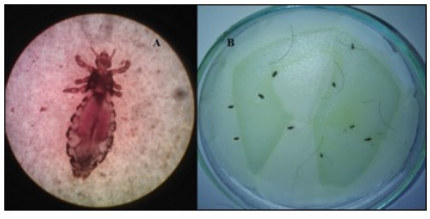
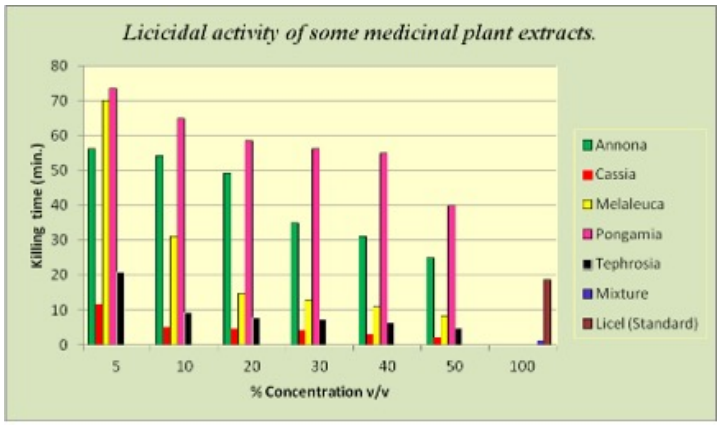


Table 3: Killing time of *Pediculus humanus capitis* adult for plant extract

Concentration	Killing Time Mean* (Min±SD)						
% v/v	Cassia obtusifolia (Mean±S. D.)	Pongamia pinnata (Mean±S.D.)	*Melaleuca alternifolia* (Mean±S.D.)	*Annona squamosa* (Mean±S.D.)	Tephrosia purpuria (Mean±S.D.)	Mixture (Mean±S.D.)	Standard (Licel) Mean±S.D
5	11.67±1.03	73.50±1.04	70.00±1.86	56.17±1.45	20.83±1.45	**1.30±0.005**	18.50±2.84
10	5.17±1.05	64.83±1.47	31.00±1.86	54.17±1.03	9.33±1.01		
20	4.67±1.12	58.50±2.79	14.67±1.03	49.17±1.45	7.83±1.47		
30	4.17±1.17	56.17±2.10	12.83±0.70	35.00±1.41	7.17±1.29		
40	3.17±1.17	54.83±1.10	10.83±1.45	31.17±1.45	6.33±0.93		
50	**2.17**±0.75	39.80±4.39	**8.50**±1.04	25.00±0.89	**4.83**±1.34		
100	--	--	--	--	--		

n* =3

Figure 4: Licicidal activity of medicinal plant extracts



Discussion

The phytochemical estimation with petroleum ether extract of seeds of Annona squamosa, Cassia obtusifolia (senna), Tephrosia purpurea (unhali), and leaves of Melaleuca alternifolia, Pongamia pinnata (karanj) includes alkaloids, glycosides, tannins, flavonoids, terpenoids and sterols were recorded. The petroleum ether extracts illustrate significant mortality time for different dilutions. In the present report, C. obtusifolia extract showed 100% mortality in 2.17±0.75 minutes at a concentration of 50% w/v and P. pinnata leaves extract showed 100% mortality at 39.80±4.39 minutes at 50% w/v concentration. Annona squamosa, Melaleuca alternifolia, and Tephrosia purpurea extract show 100% mortality at 50 % w/v concentration in 25.00±0.89, 8.50±1.04, and 4.83±1.34 minutes, respectively (Table 3 and Figure 4).

It was observed that all plant extract mixtures showed 100% mortality at the earliest, i.e. at 1.30±0.005 min as compared to standard licel which shows mortality at 18.50±2.84 min. The seed extracts of A. squamosa showed anti-head lice activity when triglyceride with one oleate ester and the crude ethyl acetate extract was used. It was diluted with coconut oil 1:1(12). The extracts of A. squamosa exhibited anti-head lice activity and reported mortality time in 10 and 31 minutes, respectively (12). Where in our study, we used petroleum ether extract of Annona squamosa and it showed 100% mortality in 25 minutes.

The ethanolic extracts from the leaves of Annona squamosa show a mortality time of 300 seconds (5 minutes) when used at 1:8 proportion with coconut oil: ethanolic extract. This indicates that they used 100% concentration whereas our study with petroleum ether seed extract at 50% concentration exhibited 25 minutes (19). This may be obvious as compared to leaf extract and concentration ratio. 100% mortality of head lice after 2 hours (120 minutes) was observed when treated with rhizome oil extracts from Hedychium spicatum (Kapur kachari) at 5% concentration (20). Whereas in our study a 5% concentration of all five selected plants extracted has given promising results less than 73.50 minutes. 100% mortality was observed with extracts of Tephrosia purpurea at 5% and 50% concentration for 20.83 and 4.83 minutes, respectively.

Pongamia pinnata petroleum ether and methanol extract have anti-pediculicidal and mild pediculocidal properties, respectively (21). In this study report Pongamia pinnata leaf extract exhibited pediculicidal activity at all concentrations with a minimum of 39.80 minutes and a maximum of 73.50 minutes at concentrations 50% and 5% respectively. The mixture of all five plant extracts exhibited promising results with a time of 1.30 minutes and licel (standard) showed 18.50 minutes for 100% licicidal activity.

Conclusion

Based on the study, it was concluded that each plant's extracts at higher concentrations were proven to cause mortality in head lice. However, a mixture of all herbal plant extracts showed mortality in a short time. The presence of phytochemical constituents like carbohydrates, tannins, proteins, alkaloids, flavonoids etc might be responsible for lice death. It was found to be safe and nontoxic in children as well.

Conflict of interest: The authors declare no conflict of interest, financial or otherwise.

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