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In Vitro Screening of Antilice Activity of *Thespesia Populnea* Fruits

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ABSTRACT

In the present study, ethanolic and aqueous fruit extracts of *Thespesia populnea* were tested against the head louse *pediculus humanus capitis*. A filter paper diffusion method was conducted for determining the potential pediculocidal activity. The results revealed that plant extracts possess excellent anti lice activity. Results were well comparable with benzyl benzoate.

Key words: *Thespesia populnea*, Anti lice activity, Filter paper bioassay, Head louse.

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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 status¹. 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 source^{2,3}. *Thespesia populnea* Linn (Family – Malvaceae) is distributed mainly along the coastal regions throughout India, often planted as avenue tree. It grows to a maximum height of 18 metres. Fruits are globose or oblong brown capsules covered with minute peltate scales, pubescent, channelled along the back⁴. The bark is often fibrous and fissured in nature with grey to brown colours. The leaves are simple, alternate, long petiolate, cordate, entire, acuminate, prominent nerves 5 – 7 with peltate scales on one or both surfaces. The flowers are yellow with purple base, slowly changing to purple on withering⁵.

This plant is astringent, cooling and anti diarrhoeal. The bark and fruits possess more curative properties. The bark is astringent and is prescribed in the Philippines in the form of a decoction for the treatment of dysentery. It is used in folk medicine as a poultice for external applications for the treatment of scabies, psoriasis and other skin ailments. The poultices prepared from fruits, flowers and leaves are also found to be useful in rheumatoid arthritis⁶.

Earlier the plant has been studied for its antibacterial, antiviral, wound healing, anticancer⁷, antisteroidogenic activity⁸ and for dermatitis⁹. Aqueous extracts of fruits of this plant are reported for its wound healing activity¹⁰.

A popular marketed hepatoprotective Ayurvedic preparation “Kamilari” consists of extracts of *Thespesia populnea* flowers, *Elettaria cardamom*, *Zingiber officinalis*, *Glycyrrhiza glabra*, *Piper longum* and Honey¹¹.

In the current study, in vitro experiment were conducted to determine the possible anti lice effect of alcoholic and aqueous extract of fruit of *Thespesia populnea* (Linn).

MATERIALS AND METHODS

Plant Material: The fruits of *Thespesia populnea* were collected from Kottayam district in Kerala, India in October 2006 and were authenticated by Mr. K G Sreekumar, Senior Research Officer, Pharmacognosy Unit, Govt Ayurveda Research Institute, Poojapura,

Thiruvananthapuram, Kerala. A voucher specimen PC-03/2006 was submitted at Academy Of Pharmaceutical Science, Pariyaram Medical College, Kannur for future reference. Dried fruits were ground to a coarse powder, passed through sieve no 24 and stored in air tight container and used for further extraction.

Ethyl alcohol extract: The shade dried powdered fruits (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The extract was concentrated in vacuo to a syrupy consistency.

Aqueous extract :The dried powders (24#) 100gm of the was taken in a 2000ml conical flask with 500ml of distilled water to which 10ml chloroform were added as a preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc was pressed and its filtrate dried in hot air oven at 45⁰C to a semisolid mass. It was stored in airtight container in a refrigerator below 10⁰C.

Collection of Head Lice

Adults of *pediculus 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 three months.

Anti Lice Activity

Ethanol and aqueous extracts of *Thespesia populneawere* tested for pediculocidal activity by filter paper diffusion method¹². All the extracts were dissolved in distilled water to obtain three different concentrations (10%, 20%, & 30%) After careful selection under a dissecting microscope, the adult's nymphs were identified and separated from nymphs. All the test organisms were divided into 10 groups (5 lice each) and were placed on a filter paper at the bottom of petridish and kept open. A 0.5ml of each test sample was poured on the test organisms and allowed to spread as a thin layer of four centimeters square. Group 1 was treated with 0.5ml distilled water and served as control. Group 2 to group 3 received 0.5ml various concentrations of ethanol and aqueous extracts of *Thespesia populnea*. Group 4 were treated with 0.5 ml of 10%, 20% and 30% benzyl benzoate 25%w/v. All the petridishes were set aside for 1 hour in a dark chamber at 26 ± 0.5⁰C and 70 ± 1% humidity¹³.

At the end of 1 hour, the dishes were taken out and applied 0.5ml of distilled water and further placed in the chamber under condition mentioned above. After 18 hours, the dishes were observed under a dissecting microscope for any possible movement of lice and absence of any

movement were considered dead¹⁴. All the treatment was triplicate.

RESULTS AND DISCUSSION

The phytochemical screening subjected to detect the presence of some secondary plant metabolites following standard procedures as shown in Table 1. The percentage yield of ethyl alcohol extract was found to be 4.12 % and aqueous extract is 6.19 %.

Table 1: Phytochemical screening of plant material *Thespesia populnea*

Phytochemical constituents	Ethyl Alcohol Extract	Aqueous Extract
Carbohydrates	+	+
Steroids	+	-
Alkaloids	+	+
Saponins	-	-
Terpenoides	+	-
Flavonoids	+	+
Tannins	+	+

(+): Present (-): Absent

Table 2: Effect of *Thespesia populnea* fruit extracts against *Pediculus humanus capitis* adults and nymphs

Groups	Test Sample	Concentration (%)	Average Mortality (%)
1.	Control	-	9.4
2.	Ethanolic extract of <i>Thespesia populnea</i>	10	56.5
		20	51.8
		30	42.3
3.	Aqueous extract of <i>Thespesia populnea</i>	10	54.6
		20	48.5
		30	38.9
4.	Benzyl benzoate 25% w/v	10	75.7
		20	67.2
		30	58.4

The use of *Thespesia populnea* ethanolic and aqueous extracts for controlling lice infestation has been authenticated from the excellent results obtained after screening various extracts for potential anti lice. Oils from natural sources, such as eucalyptus, marjoram, spearmint, peppermint, sage, rose wood, clove bud and cinnamon bark have exhibits significant pediculocidal activity in filter paper bioassays^{15,16,17,18}.

The findings of study showed excellent anti-lice activity of ethanolic extract of *Thespesia populnea* which may be due to the presence of these sterol derivatives responsible for the enhanced penetration and bio-availability 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 skin¹⁹.

Additionally, the lice was not exposed in an enclosed environment with the petridish 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^{20, 21}.

CONCLUSION

The WHO has approved the use of traditional medicines as a part of its health programmes. According to a WHO survey, 80% of the populations living in developing countries rely almost exclusively on traditional medicine for primary health care needs. As in almost all the system of traditional medicine, plants play a major role and constitute its back bone. The ethanolic extract of fruits of *Thespesia populnea* possess significant antilice activity and suggest that used in herbal formulations as pediculocidal agent.

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