

INVESTIGATION OF LICICIDAL ACTIVITY OF SOME PLANTS FROM SATPUDA HILLS

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ABSTRACT : Head louse infestation is difficult to control because of increasing lice resistance to synthetic, licicidal drugs. Licicidal activity of extract obtained from fruits of *Azadirachta indica* A. and seeds of *Annona squamosa* L. tested against the head louse *Pediculus humanus capitis*.

A filter paper diffusion bioassay method was carried out in order to determine the licicidal activity of extracts. Petroleum ether extract of each plant tested individually, showed high levels of mortality on adult lice, Both plant extract showed significant decrease in the mean time required to kill lice with 1% and 10% concentrations where as increase in the mean time was observed with 0.1% concentration when compared to 1 % lindane. *Annona squamosa* L. extract showed more potent activity than *Azadirachta indica* A. extract at all concentrations 0.1, 1 and 10% w/w. These results demonstrate the possibility of using *Azadirachta indica* A. and *Annona squamosa* L. products for controlling head lice by tribals in this area, which are difficult to control because of their resistance to the currently used anti-louse agents. The assay proved simple, effective and gave reproducible results.

Keywords: *Azadirachta indica* A., *Annona squamosa* L., Satpuda hills, Licicidal activity.

INTRODUCTION

Azadirachta indica A. Juss. (Meliaceae) is a tall, semi-evergreen tree growing wild throughout forests, along roadsides and in wastelands. Leaves are unipinnate, 20-40 cm long, crowded near the branches; leaflets are ovate-lanceolate, glabrous, serrate, acute-acuminate. Flowers white, in axillary panicles. Drupes elliptic-oblong, 1.2-2 cm long, glabrous, yellow when ripe; seed solitary, hard, glabrous, ellipsoid^{1,2}. Almost every part of this tree is used for medicinal purposes in India³. Leaves, roots, stem have been used as antimalarial⁴, antioxidant⁵. This plant have antifungal, anti-inflammatory, antibacterial, antiviral, antifilarial action and used for several other medicinal purposes in ayurvedic system of medicine⁶.

Annona squamosa L. (Annonaceae) is a small tree having height about 6 m., bark is light black, rough, longitudinally fissured. Leaves elliptic-lanceolate, 5-7×2-4 cm. Flowers solitary, green. Sepals minute. Outer petals 1.5-2 cm long, inner ones small. Fruits tubercled globose or cordate-ovoid, 5-10 cm in diameter. Seeds brown or black, smooth and having shining⁷. Seeds are known to possess insecticidal, anti-ovulatory, abortifacient and anti-implantation properties^{8,9,10}. Post-

coital anti-fertility activity is reported in the seed extract while the aerial parts are inactive¹¹.

Satpuda hills region of Maharashtra is inhabited several tribes which in their traditional system of medicine use several plants or plant-based preparations for the treatment of various ailments¹². During our course of studies on ethno medicine of this region for the plants that are used as Licicidal. We noticed that the seeds of *Azadirachta indica* and *Annona squamosa* have a wide reputation among natives of being curative for lice infections. These individual plants is being used by the tribals of Satpuda hills as licicidal agent in the form of coconut oil extract, prepared by soaking powdered seed material in coconut oil for 10-12 hours. This extract is applied on scalp once a day for three days to kill head lice.

The three major lice that infest humans are *Pediculus humanus capitis* (head louse), *Pthirus pubis* (crab louse) and *Pediculus humanus humanus* (body louse), the most common of which is the head louse. Infestations of human lice are rampant worldwide, chiefly among schoolchildren in between the age of 5-11 years both in developed as well as developing countries. Infestation rates are usually higher among girls than boys^{13,14,15,16}.

The head lice are not a main health hazard or disease vector, but can cause substantial distress due to scalp erythema, itching and probable secondary infections. Pruritis is the most widespread symptom of head lice infestation. Bite reactions, excoriations, cervical lymphadenopathy, and conjunctivitis are also common manifestations¹⁷. The adult louse is 2 to 3 mm long and usually light gray, although color may differ. The female lives up to 3 to 4 weeks. After mating, an adult female louse lays one to six eggs a day for up to one month until death. The eggs incubated by body heat, hatch in 10 to 14 days. Once the eggs hatch, nymphs leave the shell casing, breed for about 9 to 12 days, and develop into an adult lice and mate, and then females lay eggs. If not treated, this cycle may repeat itself every 3 weeks. While living on the head, the louse feeds by injecting small amounts of saliva and taking tiny amounts of blood from the scalp every few hours. The spread of head lice is thought to occur mainly through direct head to head (hair to hair) contact. Transmission can also occur through personal belongings of an infested individual (combs, brushes, hats)¹⁸. Over the last two decade despite the availability of many over-the-counter (OTC) licideal products, research indicating resistance to these OTC licideal products¹⁹.

EXPERIMENTAL

Ripe fruits of *Azadirachta indica* A. Juss. (Meliaceae) and seeds of *Annona squamosa* L. (Annonaceae) were collected from Karvand road Shirpur, Dist- Dhule (MS). These fruits, seeds and plants were identified and authenticated by Prof. Dr. D. A. Patil, Department of Botany, S. S.V.P.S. society's Dr. P. R. Ghogrey Science College, Dhule. This fruits and seeds were dried in sunlight, pulverized, passed through sieve no. 40 and used for extraction.

Extraction

Above powder of *Azadirachta indica* and *Annona squamosa* were exhaustively extracted in Soxhlet apparatus by using petroleum ether (40-60°C). Extractive value of *Azadirachta indica* and *Annona squamosa* were found to be 33.4% w/w and 28.2 % w/w respectively.

Test Solutions

0.1, 1, 10 % w/w Test solutions of *Azadirachta indica* and *Annona squamosa* extracts were prepared separately in coconut oil.

Standard Solution

1 % w/w lindane solution prepared in coconut oil.

Experimental Organism

Human head lice *Pediculus humanus capitis* (Trichodectidae) were collected from tribal children between the age of 3-12, with the approval of their guardians, residing in tribal pada near Boradi town in Dhule district. The insects were collected by combing the children scalps. The children had not been treated with any licideal solution for at least the preceding month, using only the louse comb. After collection the lice were pooled and held on human hair strands in petri

dishes. The in-vitro tests were started within 1 h after collection. In an earlier observation independent of present study the lice were found to remain live for 24-48 hours when removed and kept away from host body.

For testing the licideal activity, a filter paper diffusion bioassay was made. After careful selection of lice under a dissecting microscope, a filter paper discs (Whatman No 1; 9-cm diameter) coinciding with internal diameter of petri dish were cut and placed in petri dishes, 0.25 gms of each test solution was spread over the lice and filter paper by using brush in each group. The 0.1, 1 and 10% solutions of *Azadirachta indica* extract and *Annona squamosa* extract were tested for the licideal activity. The dilutions were prepared in coconut oil as base. Negative control lice were placed directly on the filter paper spread with only coconut oil. The 1 % w/w lindane solution in coconut oil was simultaneously run as a positive control, 1 % lindane topical lotion is commonly used synthetic insecticide to treat lice infestation hence licideal activity of these extracts were compared with lindane²⁰. The number of lice were 10 in each 0.1, 1, 10 % w/w test solutions of *Azadirachta indica* and *Annona squamosa* extract group, negative control group and positive control group. The criteria used for survival of lice were extremely strict. If any minor signs of life, such as movements of antennae or minimal leg movements were observed (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 test was done in duplicate and average considered^{21,22}.

RESULTS AND DISCUSSION

Both plant extract showed significant decrease in the mean time required to kill lice with 1% and 10% concentrations where as increase in the mean time was observed with 0.1% concentration when compared to 1 % lindane (Table.1). *Annona squamosa* L. extract showed more potent activity than *Azadirachta indica* A. extract at all concentrations of 0.1, 1 and 10% w/w. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of *Azadirachta indica* A. and *Annona squamosa* L. extracts for controlling head lice by tribals in this area, which are difficult to control because of their resistance to the currently used anti-lice agents.

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Table 1: Licicidal activity of *Azadirachta indica* and *Annona squamosa* extract

Group	Treatment	Mean Time in Minutes \pm S.E.M.
Group 1	0.1 % w/w <i>Azadirachta indica</i> extract test solution	116* \pm 1.15
Group 2	1 % w/w <i>Azadirachta indica</i> extract test solution	60* \pm 1.72
Group 3	10 % w/w <i>Azadirachta indica</i> extract test solution	31* \pm 1.31
Group 4	0.1 % w/w <i>Annona squamosa</i> extract test solution	107* \pm 1.59
Group 5	1 % w/w <i>Annona squamosa</i> extract test solution	57* \pm 1.76
Group 6	10 % w/w <i>Annona squamosa</i> extract test solution	28* \pm 1.19
Group 7	1 % w/w Lindane solution (Positive control/standard)	54* \pm 1.95
Group 8	Plain coconut oil (Negative control)	-----

n = 10 in each group.

Values are Mean time in minutes at which lice were considered dead \pm S.E.M.

***The data was analyzed by Student's t test, p value < 0.01 was considered statistically significant.**

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