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Pediculocidal Activity of *Lowsania inermis* L. against the Head Lice *Pediculus humanus* *capitis* De Geer (Phthiraptera: Pediculidae)

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ABSTRACT: In the present study the dried leaf powder of *Lowsania inermis* was extracted with acetone and evaluated against the first, second and third nymphal stages of *Pediculus humanus capititis*. The *L. inermis* extract was tested for pediculocidal activity by filter paper diffusion method. Samples of 1, 2, 3, 4 and 5ml from stock solution were dissolved in 100ml of distilled water to make different concentrations like 100 to 500ppm and each concentration was applied to filter paper (whatman No.1) for antipediculus bioassay. After 18 hours the mortality was calculated. From the morality values observed LC₅₀ and LC₉₀ values were 122.959, 312.539 ppm for first nymphal stage; 171.151, 370.149 ppm for second nymphal stage; 214.458, 369.575 ppm for third nymphal stage respectively. The phytochemical constituents of *L. inermis* was evaluated by GC-MS shows the presence of 20 different compounds with various molecular weight and retention time. The results obtained suggest that the acetone leaf extract of *L. inermis* promising as licidical activity against *P. humanus capititis*. The secondary metabolites which are present in *L. inermis* extract were jointly or synergistically causes the licidical effect against *P. humanus capititis*.

KEYWORDS: *Lowsania inermis*, *Pediculus humanus capititis*, licidical effect and phytochemicals

I. INTRODUCTION

Pediculosis is a skin infestation caused by hematophagous lice, the most common of which is the head louse, *Pediculus humanus capititis* De Geer. Infestations of human lice are prevalent worldwide, especially among school children in both developed and developing countries [1]. According to Meinking *et al.* [2]the number of cases of head and body lice infestation has been estimated to be higher than 100 million. *P. humanus capititis* infections cause skin irritation, pruritus and sleep loss, as well as occasional secondary bacterial infection from scratching [3]. Although the symptoms are relatively mild, infestation by *P. humanus capititis* has resulted in various social, mental and economic problems. The control of human head lice worldwide depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphorus (malathion), carbamate (carbaryl), pyrethrin, pyrethroid (permethrin and 6-phenoxyphenyl) and avermectin (ivermectin-originated from *Streptomyces avermitilis*) insecticides [4]. Their repeated use has often resulted in the development of levels of resistance to the most commonly used pediculicides have caused multiple and excessive treatments, fostering serious human health concerns [5]. 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 [6].

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The well-documented information for traditional head lice treatment, either with ethnobotanicals or other methods, is rather scarce. Products used in popular medicine include vinegar, formic acid, isopropyl alcohol, olive oil, mayonnaise, melted butter and petroleum jelly [7]. How these products work can only be guessed and none have been proved to effectively kill lice. Acids may act upon the nits by softening the protective sheath that covers and attaches the egg to the hair [8]. Oily products may affect the respiratory spiracles of the lice or slow the insects making it easier to physically remove them with a fine comb [7]. These problems have highlighted the need for the development of selective *P. humanus capititis* control alternatives, particularly with fumigant action and application to human hair. Plant essential oils have been suggested as an alternative source of materials for insect control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents for foods and beverages [9]. Because of this, much effort has been focused on plant essential oils or phytochemicals as potential sources of commercial head lice control agents [10] and [11]. Little work has been done on pediculicidal activity of plant essential oils, although insecticidal activity of essential oils has been well described by Isman [9].

Genus *Lawsonia* bears one species, *L. inermis* (Henna, Mhendi, Shudi, Madurang, Mendi, Manghati, Madayantika and Goranti) [12] till date, having different synonyms as *alba* and *spinosa* belonging to family Lythraceae. It is a biennial dicotyledonous herbaceous shrub. A native of North Africa and South-West Asia, the plant is now widely cultivated throughout the tropics as an ornamental and dye plant. Although this plant has been widely used in various symptoms and diseases, however few pharmacological studies have been reported like antidiabetic, immunomodulatory effect, hepatoprotective activity, antioxidant effect, antifungal activity, antiviral activity, antitrypanosomal activity, antiparasitic activity, larvicidal, molluscicidal activity, antifertility activity, analgesic activity, anti-inflammatory activity, cytotoxic activity, antisickling activity, abortifacient activity, enzymes inhibitory activity, nematicidal effect, anticoagulant effect, wound healing effects and protein glycation inhibitory activity [13]. But the report for licidical effect of *L. inermis* not reported by any researchers. Hence in the present study an attempt has been made to assess the pediculicidal activity of *L. inermis* against *P. humanus capititis* and the phytochemical constituents responsible for licidical effect was evaluated by Gas Chromatography Mass Spectrometry (GC-MS) analysis.

II. MATERIALS AND METHODS

2.1. Collection and maintenance of head lice

Pediculus humanus capititis were collected from school going children between the age group of 8-12 by combing using a clean, sharp teeth white comb. After careful collection of lice, nymphs and nits, they were kept in sterile petri plates containing human hair strands. The lid was loosely sealed by parafilm wax to permit ventilation and immediately transported to the research laboratory. The in-vitro tests for head lice were started within 1 hr 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.

2.2. Antilice assay

In the present study *L. inermis* was tested for pediculocidal activity by filter paper diffusion method[14] Samples of 1, 2, 3, 4 and 5ml from stock solution were dissolved in 100ml of distilled water to make different concentrations like 100 to 500ppm and each concentration was applied to filter paper (whatman No.1) for antipediculus bioassay. For control group filter paper was treated with distilled water and respective concentrations of acetone only. After the evaporation of solvent from filter papers by drying in air, two filter papers were placed in the bottom of each petri dish provided with lid. A soaked cotton plug was placed in each petri dish to maintain their moisture content. Twenty active lice were put into each petri dish on filter paper, covered and placed in dark. 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. Extract was tested for pediculocidal activity by filter paper diffusion method [15]. After 18 hrs the dishes were observed under a dissecting microscope for any possible movement of lice and absence of any movement were considered as dead[16].

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2.3. Phytochemical analysis by GC-MS (Gas Chromatography-Mass Spectrometry)

Dried plant extract was dissolved in methanol and analyzed using GC-MS SHIMADZU QP2010 instrument with GC-MS solution version 2.53SU3 software. The sample was analyzed with Elute – DB-5M column. Initially oven temperature was maintained at 70° C for 2.0 min, and the temperature was gradually increased up to 300° C at 10.0/35.0 min and 4.0 µL of sample was injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as eluent. The flow rate of helium gas was set to 1.5 mL/min. The sample injector temperature was maintained at 260°C and the split ratio is 20 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectrum was recorded across the range 40-1000 m/z for the duration of 35 minutes. Identification of components was based on comparison of their mass spectra. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with (NIST Library 2008 WILEY8, FAME).

III. RESULTS AND DISCUSSION

In the present study the acetone leaf extract of *L. inermis* evaluated against the first, second and third nymphal stages of *P. humanus capititis* by tissue paper diffusion assay. After 18 hours the mortality was calculated. From the morality values observed LC₅₀ and LC₉₀ values were 122.959, 312.539 ppm for first nymphal stage; 171.151, 370.149 ppm for second nymphal stage; 214.458, 369.575 ppm for third nymphal stage respectively. Elucidation of the modes of action of pediculicidal natural products and insecticides is of practical importance for insect control because it provides useful information on the most appropriate formulation and delivery means. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids, and exhibit fumigant activity [11]. Plant extract and essential oils have potential as natural products for *P. humanus capititis* control because some of them are selective, have little or no harmful effects on non-target organisms and can be applied to humans in the same way as other conventional insecticides [10]. Pediculicidal activity has been reported for some essential oils such as aniseed, cinnamon leaf, thyme red, tea tree, and nutmeg oils [17]; neem oil [18] and anise and ylang ylang oils [10].

Alkaloids are chemicals which are produced by plants are nitrogenous in nature. These are chemical poison of plants, are heterocyclic in nature and have direct effect on nervous system of animals and cause desolation. Therefore these alkaloids are named as nerve poisons [19]. Many scientists are now paying their attention to find out such type of plant toxicants, which are environmentally safe and can control these pests in much better way. Study carried out on school children revealed that 20% petroleum ether extract of custard apple seeds killed 95.3% of head lice [20]. Another study revealed that Oils from natural plant sources, such as eucalyptus, spearmint, peppermint, cinnamon bark, sage, rosewood, clove bud, and marjoram have exhibited significant pediculocidal activity in filter paper bioassays [11]. 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 [21]. Plant extracts has been noticed for its safe and effective use, and appearance of resistance patterns were minimal due to its different mode of action [22].

In the present investigation phytochemical constituents of *L. inermis* was evaluated by GC-MS shows the presence of 20 different compounds with various molecular weight and retention time (Table.2; Fig.2). There are many researchers investigated the phytochemicals which are presented in *L. inermis*. It has naphthoquinone derivatives, xanthones, coumarin, fatty acids, amino acids and other constituents. Naphthoquinone fraction obtained from leaves of *L. inermis* showed significant immunomodulatory effect. Quinonic compounds from henna were studied invitro for antimicrobial properties. Lawsone isolated from the leaves of *L. inermis* has shown significant anti-fungal and antibiotic effect [23]. The results obtained suggest that the acetone leaf extract of *L. inermis* promising as licidical activity against *P. humanus capititis*.

The secondary metabolites which are present in *L. inermis* extract were jointly of synergistically causes the licidical effect against *P. humanus capititis*. The present study concluded that the henna leaf paste is used for hair

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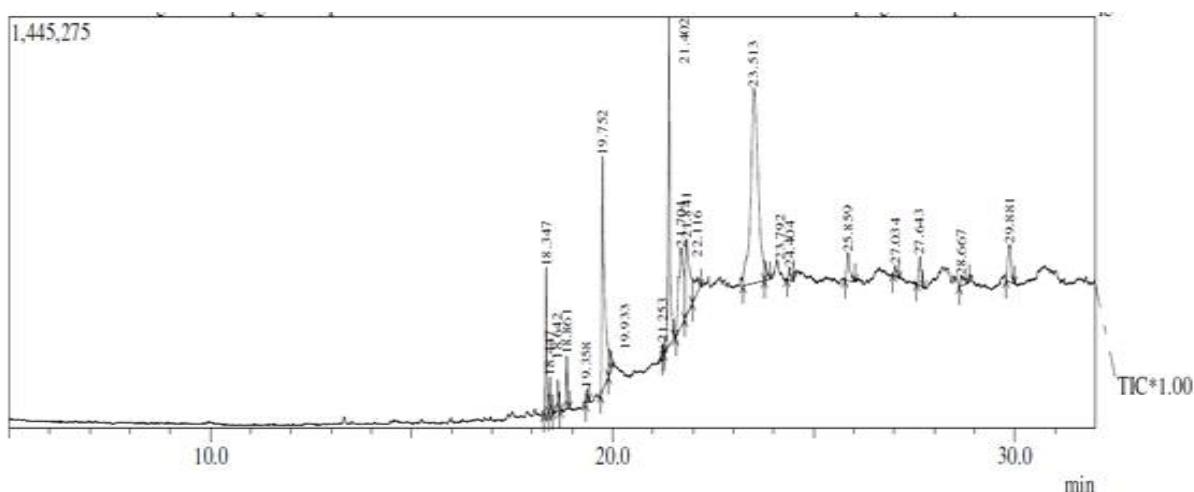
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colouring agent and simultaneously the phytochemicals which are present in the leaf will control the head lice *P. humanus capititis*. Lice infestations are common worldwide whereas most of the currently available synthetic pediculicidal agents in the market are cost effective and expensive. Though all of the synthetic pediculicidal agents act efficiently against *Pediculus humanus capititis*, but these products are found to be sometimes neurotoxins also [24]. Hence non toxic alternative options are needed for treatment of pediculosis. Natural source remains less or non toxic and less cost effective. Natural extracts from medicinal plants always act as a rich source for treatment of various diseases and disorders of human system. Recently they are gaining much more importance because of their broad range of pharmacological actions [25]. More than 1000 plants species have been described from many areas which have chemicals components in their seeds, stems, roots, leaves and flowers against insect pests. Among these plants, only few plants have been used to control insects practically on commercial scale in the last few decades.

Table. 1. LC₅₀ and LC₉₀ values of acetone leaf extract of *L. inermis* on I-II nymphal stages of *P. humanus capititis*

S. No	Developme ntal stages	Mortality					LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	Regression equation	Chi- Squa re (χ ²)
		100 ppm	200 ppm	300 ppm	400 ppm	500 ppm				
1.	First nymphal stage	4.8±1.5	6.8±0.9	8.2±1.7	10±0.0	10±0.0	122.959 (72.716- 184.165)	312.539 (248.876- 477.195)	Y= -0.831+0.168X	8.609
2.	Second nymphal stage	3.6±1.3	5.4±1.25	7.8±0.5	9.2±0.8	10±0.0	171.151 (144.412- 193.434)	370.149 (341.654- 407.843)	Y= -1.102+ 0.157X	3.132
3.	Third nymphal stage	2.2±0.8	3.8±1.30	7.6±1.1	9.4±0.8	10±0.0	214.458 (196.244- 231.472)	369.575 (345.638- 400.152)	Y= -1.772+0.173X	4.661

Fig. 2. Gas Chromatogram of acetone leaf extract of *L. inermis*



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Table: 2. GC-MS analysis of acetone leaf extract of *L. inermis*

Peak	R.Time	I.Time	F.Time	Area%	Height %	A/H	Name of the compound
1	18.347	18.300	18.400	4.53	10.52	2.32	2-Hexadecen-1-OL,3,7,11,15-tetrame
2	18.447	18.400	18.508	1.37	2.56	2.87	2-Pentaecanone,6,10,14-trimethyl-\$\$ Hexat
3	18.642	18.508	18.700	0.97	2.25	2.32	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	18.861	18.700	18.917	1.43	3.82	2.01	2-Hexadecen-1-OL,3,7,11,15-tetrame
5	19.358	19.333	19.408	0.41	0.99	2.22	cyclopentanetridecanoic acid
6	19.752	19.700	19.900	14.42	16.90	4.59	n-Hexadecanoic aci \$\$ Hexadecanoic acid \$\$
7	19.933	19.900	19.975	1.13	1.58	3.83	2-(Isobutoxycarbonyl)benzoic acid \$\$ componends
8	21.253	21.225	21.275	0.33	0.81	2.21	Cyclopropaneoctanoic acid, 2-octyl-, methyl
9	21.402	21.275	21.542	13.41	23.70	3.04	Phytol isomer
10	21.704	21.567	21.792	9.11	5.68	8.63	Dichloroacetic acid, tridec-2-ynyl ester
11	21.841	21.792	21.983	7.31	5.49	7.17	Octadecanoic acid \$\$ Stearic acid \$\$ n-Octadecanoic
12	22.116	21.983	22.183	2.23	1.08	11.0	1,2,5,6,7,8,9,10,11,12,13,14,15,16-tetrade
13	24.404	24.333	24.492	0.66	0.88	4.06	Eicosane \$\$ n-Eicosane \$\$ Icosane # \$\$ n-Icosane
14	23.513	23.242	23.775	33.91	14.07	12.96	Vitamin E \$\$ 2H-1-Benzopyran-6-ol, 3,4-dihydro
15	23.792	23.775	23.925	0.99	1.37	3.89	3-Ethoxy-1,1,1,5,5-hexamethyl-3-(trimethyl
16	25.859	25.775	26.033	1.97	2.03	5.20	Tetrapentacontane
17	27.034	26.975	27.117	0.51	0.77	3.56	Bis(2-ethylhexyl) phthalate \$\$ Phthalic acid,
18	27.643	27.567	27.733	1.61	2.09	4.15	Nonacosane \$\$ n-Nonacosane \$\$
19	28.667	28.617	28.867	0.83	0.71	6.29	Benzeneacetaldehyde, alpha.-(phenylmethylen)- \$\$ (2Z)-2-Benz
20	29.881	29.783	29.992	2.87	2.69	5.73	Hexatriacontane \$\$ n-Hexatriacontane \$\$
			Total	100%			

IV. CONCLUSION

The head lice, *Pediculus humanus capitinis* (Phthiraptera:Pediculidae), is an obligate ectoparasite of humans that causes pediculosis capitis, a nuisance for millions of people worldwide, with high prevalence in children. Pediculosis capitis has been treated by methods that include the physical removal of lice, various domestic treatments and conventional insecticides. None of these methods render complete protection, and there is clear evidence for the evolution of resistance and cross-resistance to conventional insecticides. Non-toxic alternative options are hence needed for head lice treatment and/or prevention, and natural products from plants. In the present study a new effort has been made for controlling the human head lice by applying the *L. inermis* extract. The plant powder is used for natural hair dye. Mean time head lice can be controlled without any additional need. The phytochemicals which are responsible for licecidal effect were evaluated by Gas Chromatography Mass Spectrometry (GC-MS).

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