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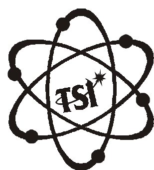

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The Foliar Fatty Acids In The Weed, *Ludwigia Adscendens* L.

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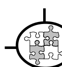
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ABSTRACT

The foliar fatty acid fractions and their relative concentrations in the weed, *Ludwigia adscendens* L. were determined by TLC and GC-FID analyses of methyl esters in the n-hexane extract of mature leaves. The lipids content was 5.74% of the mg/g dry leaf tissue. Fatty acids identified were palmetic acid (C_{16}), oleic acid (C_{18}) and stearic acid (C_{18}) having 65.57, 4.85 and 10.79% concentrations, respectively. The identity of the peaks 1, 2 and 4 in the chromatogram could not be obtained, but these indicated 6.88, 6.09 and 5.82% concentrations in the mature weed leaf, respectively. © 2007 Trade Science Inc. - INDIA

KEYWORDS

Ludwigia adscendens;
TLC;
GC-FID;
Foliar fatty acids.

INTRODUCTION

A few studies have described leaves and flowers of Ludwigia species as having some important pharmaceutical and allelopathic properties in their natural or unprepared state[2,3] but the lack of proper analysis and identification of the chemical compounds, particularly fatty acid components in Ludwigia has somewhat prematurely weakened these accounts. The shiny and mature (4-8 weeks old) leaves of the water primrose, *Ludwigia adscendens* L. (Myrtales: Onagraceae), a troublesome weed inhabiting the marshland and rice fields in India and elsewhere, attracted the important biocontrol agent, *Altica cyanea* (Coleoptera: Chrysomelidae) in large numbers for defoliation[12,15]. Previous studies on some volatile hydrocarbons, such as long-chain alkanes and less volatile triterpenes, isolated from cuticular waxes of the mature leaves of this weed, and tested to find out if any attraction could be produced for this insect or tested to find out act of feeding could be caused to this insect in the olfactory bioassay, yielded results

useful to some extent[4,5]. But neither the chemical composition nor the concentration of the lipid components, mainly of the fatty acid fractions present in cuticular waxes of this weed, could be assessed to date. The present study is an attempt for the first time to analyze and identify the fatty acid fractions, and to determine their relative concentrations in the cuticular layers of mature L. adscendens leaves.

EXPERIMENTAL

Lipids extractione

The mature (4-8 weeks old) *L. adscendens* leaves, found to be in their final satage of blade expansion and ideal for defoliation by the potential biocontrol agent, *A. cyanea* in the field[12] have been considered as the model for extraction of soluble cuticular lipids and chemical analysis in this investigation. The leaves were randomly harvested from weeds growing in the rice fields adjacent to the Post-graduate Departments of the University of Burdwan at Golapbug (23°16′N, 87°54′E) during June-September 2005. Fresh leaves were initially washed with clean distill water and dried on paper toweling. For extraction of epi- and intra- cuticular soluble lipids, whole leaf blades were initially homogenized in 20 mL $CHCl_{3:}$ MeOH (1:2 v/v) mixture at room temperature for 10 min in a cell homogenizer, and cuticular lipids were extracted following the standard methods outlined by[7]. The lipids extracted were dried in a desiccator and weighed in an electro-balance (± 0.01 mg). The determination was repeated three times, and the yield (with variations from ± 0.1 to ± 1%) was expressed as percent of the mg/g dry leaf tissue.

Chemical analysis

Additional 100 g fresh and paper toweled, whole leaf blades were dipped in 2 L n-hexane in a tightly plugged conical flask (3L) and shaken once daily for 21 days at room temperature, and the crude extract was passed through Whatman No.41 filter paper (Maidstone, UK). The preparation was slowly evaporated to dryness under reduced pressure and the dried sample was mixed in 50 mL CH_3OH: $HCIO_4$ (95:5). The mixture was warmed in a hot-water bath at 50-60°C temperature, and the compounds were extracted with 50 mL petroleum ether in a separating funnel. The upper petroleum ether layer was collected in a 250 mL glass conical flask, whilst the residue was re-extracted twice with 2×50 mL petroleum ether. The supernatants were pooled and filtered through Whatman No.41 filter paper. The filtrate was dried over 50g anhydrous Na_2So_4. Prior to gas chromatography (GC), 2mL petroleum ether was added to the ester every time.

The esterified fatty acids were initially separated and purified by thin layer chromatography (TLC) using n-hexane: ethyl acetate (1:1) as the mobile phase. The TLC was done on the plate having a Silica gel G (Sigma, St. Louis, Mo, USA) layer (0.5 mm thickness), which was prepared by using a Unoplan (Shandon, London) coating apparatus. The fatty acid fractions were analyzed directly on a Hewlett Packard gas liquid chromatograph (HP-GLC; Palo Alto, CA, USA; model 6890) fitted with a HP-5 capillary column (30m long and 0.32 mm i.d.) and a flame ionization detector. The GC was carried out temperature programmed injection at 150°C, increasing by 4°C min^{-1} to 250°C, and oven 5 min at 250°C for. The peaks obtained were compared in retention times with authincated standards and literature data. The carrier was nitrogen at a flow rate of 16.5 mL/min. All solvents used were of analytical grade and purchased from E. Merck (Mumbai, India).

RESULTS AND DISCUSSION

Immersing 1g *L. adscendens* whole leaf blades into CH_3OH: $HCIO_4$ (1:2 v/v) mixture yielded 5.74% cuticular lipids (TABLE 1). This is in contrast with the fatty acid contents in higher plants that comprised up to 7% of the dry weight of the leaf tissue. Nevertheless, the concentration of the identified and unidentified fatty acid fractions ranged between 81.21 and 18.79% of the total amount of fatty acids, respectively. The concentration of palmetic acid was maximum (65.67%), whilst the concentration of oleic acid was minimum (4.85%) in the cuticular lipids of this weed.

The identity of fatty acid fractions produced on esterification of the n-hexane extract of cuticular lipids of mature *L. adscendens* leaves was established

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TABLE 1: Lipids content (%) and relative concentrations of the identified and unidentified fatty acid fractions in mature *L. adscendens* leaves

Fatty acid fractions	Retention time (min)	Relative concentration (mol %)	Lipids content (% of mg/g dry leaf tissue)
Identified			**5.74***
Palmetic acid - peak 3	13.328	65.57	-
Oleic acid - peak 5	18.396	4.85	-
Stearic acid - peak 6	19.254	10.79	-
Unidentified			
Peak 1	8.193	6.88	-
Peak 2	12.696	6.09	-
Peak 4	18.174	5.84	-

*measured as Folch *et al.* (1957)

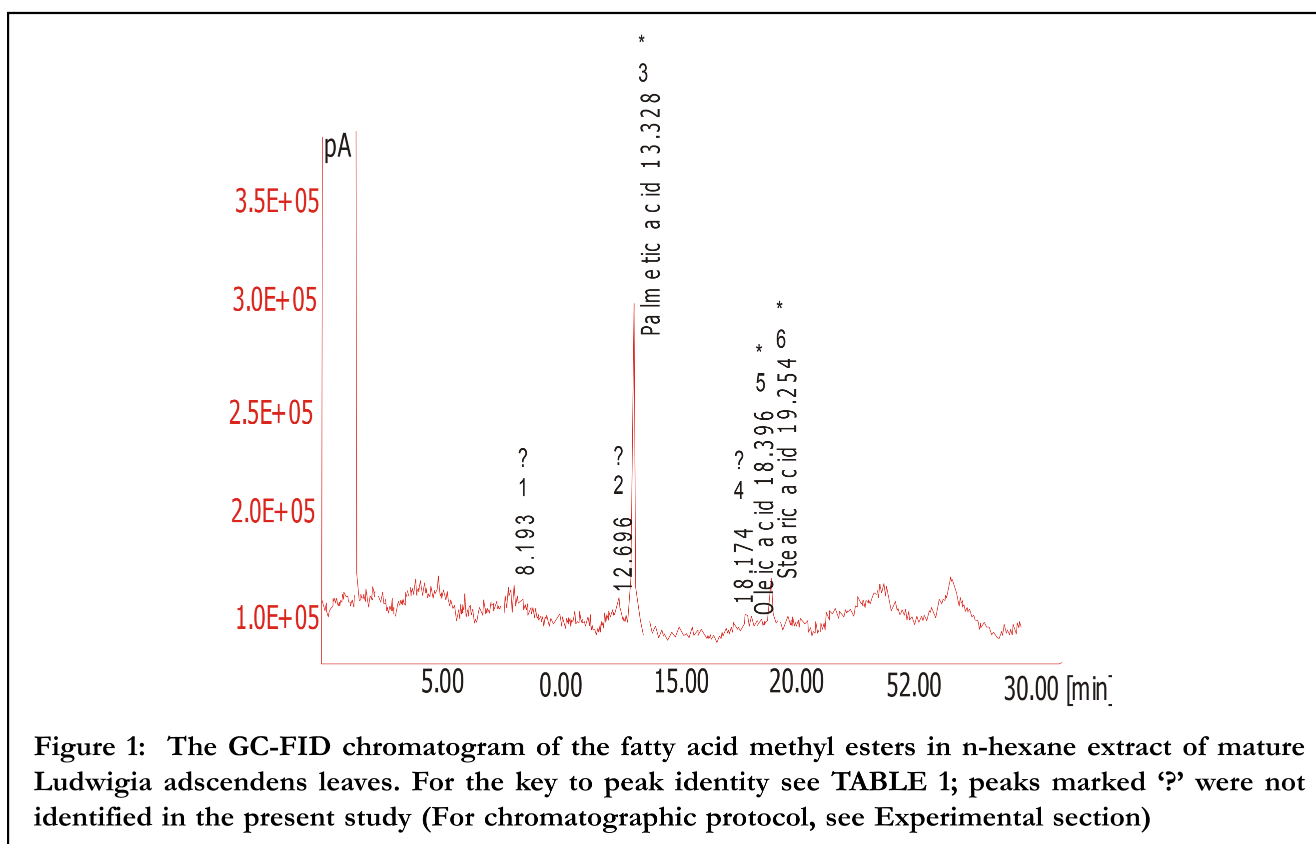

Figure 1: The GC-FID chromatogram of the fatty acid methyl esters in n-hexane extract of mature Ludwigia adscendens leaves. For the key to peak identity see TABLE 1; peaks marked '?' were not identified in the present study (For chromatographic protocol, see Experimental section)

in the GC-FID chromatogram (Figure 1). Three fatty acids peaks, namely peak 3 - palmetic acid (C_{16}), peak 5 - oleic acid (C_{18}), peak 6 - stearic acid (C_{18}) were identified, whilst the identification of other three peaks, namely 1, 2 and 4 in the GC-FID could not be obtained, probably because of higher degrees of unsaturation with some unusual fatty acids.

CONCLUSIONS

Although a number of fatty acids are known in leaves, flowers and seeds of plants[1,4,8,9,10,11,13], the identification of soluble cuticular lipids in the weed, particularly the fatty acid components in this weed, would make this chromatographic analysis useful to a great extent. Folivorous insects (e.g. *A.cyanea*) among others may probe the chemical nature of the cuticular waxes and use the candidate fatty acids to

recognize this weed host for feeding and oviposition [4,14]. The fatty acids identified in this investigation were either saturated or simple unsaturated compounds of C_{16} or C_{18} chain length[8] maintained that palmetic acid, a C_{16} acid, was the major saturated acid in the leaf of higher plants. Consistent with this data, palmetic acid, a C_{16} acid, was the major saturated acid in the present investigation and found in quantity in cuticular waxes of the mature leaf of this weed. In contrast, oleic acid was the lesser unsaturated C_{18} acid and comprised 4.85% of the fatty acids in cuticular waxes of the mature leaf of this weed. Furthermore, the composition of fatty acids extracted from photosynthetic spinach (*Spinacia oleracea*) leaves showed a significant difference in amounts of $C_{18:2}$ and $C_{18:3}$ acids and $C_{16:0}$ dominated the leaf lipids[6]. It remains to be seen how far the fatty acids identified in this weed are repeated for other weed species growing in rice-weed systems, or how the cuticular lipids would serve as attractants for contact-probing insects including *A. cyanea* for defoliation in the field.

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