



## Identification of Omega 3 Fatty Acid Ester and Antimicrobial Activity of Yellow Ornamental Lantana Flowers

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### ABSTRACT

The current work on Yellow Ornamental Lantana flowers focus on investigating its possible antimicrobial properties, the presence of active alkenes and long chain fatty acid molecules. It was observed to have effective antimicrobial activity against *S. aureus*, *P. mirabilis*, *P. aeruginosa*, *B. cereus* and *E. coli*. The presence of Erucamide and Cis-5, 8,11,14,17-eicosapentaenoic acid, tert-butyl dimethylsilyl ester (a derivative of omega-3- fatty acid, eicosapentaenoic acid) was reported by the GC-MS analysis of the methanolic extract of the flowers.

**Keywords:** Eicosapentaenoic acid, Erucamide, GC-MS, Omega 3 fatty acid.

### INTRODUCTION

Lantana is a large genus of perennial flowering plants consisting of about 150 species.<sup>1</sup> They are native to tropics of America and Africa but also exists as an introduced species in many parts of the world.<sup>2</sup> Many of the species of Lantana are grown as an ornamental plant throughout the world.<sup>3</sup> They are known for their beautiful colored flowers. *Lantana camara* is the most widespread species of this genus.<sup>4</sup> It has been regarded as one of the most noxious weeds in the world.<sup>5-7</sup> However it has also been listed as one of the most important medicinal plants of the world<sup>8</sup> and cultivated as a garden plant. Anti-fungal<sup>9</sup>, antimicrobial<sup>10</sup> and even termiticidal<sup>11</sup> effect have been reported previously. It has also been found effective against some of the multi drug resistant bacteria isolated from the ICU.<sup>12</sup> Several biologically active compounds like terpenoids, steroids, and alkaloids have been reported as the major chemical constituent in the extracts of different part of *Lantana camara*.<sup>13-16</sup> The biochemical parameters like lipids, carbohydrates and proteins have been found to be almost similar in *Lantana camara* plants species with yellow, lavender, red and white flowers.<sup>17</sup>

Some new hybrids of Lantana have been developed to be used as ornamental plants. Yellow Ornamental Lantana (YOL) is one such sterile hybrid of Lantana which is being used widely.

### MATERIALS AND METHODS

#### Plant extract

The flowers of the YOL were collected from VIT University, Vellore campus. It was recognized using Identification guide: Lantana flowers guide.<sup>18</sup> They were shade dried and ground to form uniform powder. The ethanolic extract was prepared by adding 20gm of dried powder to 100 ml of 70 % ethanol by refluxing for 5 hrs.



Figure 1: Yellow Ornamental Lantana (YOL) flowers.

#### Antimicrobial assay

The antibacterial activities of the ethanolic (70 %) extracts of the flowers of the Yellow Ornamental flower were evaluated by the agar well diffusion method.<sup>19</sup> The bacterial strains that had been incubated overnight were used for the assay. A sterile "L rod" was used to evenly spread the bacterial inoculum over the entire surface of a sterile Mueller Hinton agar plate.<sup>20</sup> Wells were punched on the seeded plates using a sterile borer (8mm) and the plates were allowed to dry for 15 min. The two concentrations, 20gm/100ml and 10gm/100ml were tested against the five bacterial species namely *S. aureus*, *P. mirabilis*, *P. aeruginosa*, *B. cereus* and *E. coli*. Ofloxacin was used as positive control, whereas ethanol 70% was taken as negative control. The plates were incubated overnight at 37°C and the antibacterial activity was determined by measuring the zone of inhibition (mm).

#### GC-MS analysis

The flower powder was dissolved in 80% methanol and left for 24 hrs. on shaker(at 110rpm) for phytochemical extraction. The methanolic extract was then filtered using Whatman No. 1 filter paper. The filtered extract was dried at 45 -50°C in oven to obtain solid crystal. The crystal was subjected to GC-MS analysis. The analyses of the volatile compounds were carried out on a Perkin Elmer GC/MS

system (GC Model clarus 680, MS clarus 600, EI). Column = Elite-5 MS (30.0m, 0.25mmID, 250µm df) was coupled to the mass spectrometer. The carrier gas was helium (1 mL/min). The temperature program used: initial temperature was 60°C held for 2 min, ramp 10°C/min to 300°C, hold for 6 minutes. The injection port temperature was 250°C. Total run time was 32 minutes. Mass Condition (EI) was Solvent Delay = 2.00 min, Transfer Temperature = 230°C, Source Temperature = 230°C and Scan: 50 to 600Da. The individual constituents were identified by their identical retention indices, by comparing their mass spectra with either the known compounds or with the MS data bank.

## RESULTS AND DISCUSSION

### Antimicrobial

The antimicrobial effect of YOL flower has been discussed in the table below (Table 1). It was found that the extracts possess strongest antimicrobial activity against *S. aureus* and *B. cereus*. For all the microbes except *E. coli*, the zone of inhibition was found to be greater than the positive control at 20mg/100ml concentration. Most bacteria form biofilm which is resistant to commonly used antibiotics.<sup>21</sup> So investigating different plants (especially weeds) for their possible antimicrobial action will be a more noble approach to help proper exploitation of these weeds and prevent their harmful environmental impact.

**Table 1:** Antimicrobial activity of ethanolic extract of leaf against the listed microbes below

Microbes	Zone of inhibition (mm)		
	A (20mg/100ml)	B (10mg/100ml)	Control/Ofloxacin 5mcg
<i>B.cereus</i>	11.5	7.5	5.0
<i>E.coli</i>	4.5	2.5	13.0
<i>P.aeruginosa</i>	6.5	3.5	4.0
<i>P.mirabilis</i>	8.5	5.5	7.0
<i>S.aureus</i>	10.5	5.5	5.0

**Table 2:** Major compounds reported by GC-MS analysis

Major Compounds	Molecular formula	Molecular weight
3-[(3-fluorophenyl) carbamoyl] -1,2,2-trimethylcyclopentane-1-carboxylic acid	C <sub>16</sub> H <sub>20</sub> FNO <sub>3</sub>	293.33
1,5-Diphenyl Hex-3-ene	C <sub>18</sub> H <sub>20</sub>	236.35
Cis-5,8,11,14,17-eicosapentaenoic acid, tert-butyl dimethylsilyl ester	C <sub>26</sub> H <sub>44</sub> O <sub>2</sub> Si	416.71
(Z)-docos-13-enamide or Erucamide	C <sub>22</sub> H <sub>43</sub> NO	337.58

### CONCLUSION

The ester of Omega 3 fatty acid and 3 other active compounds were reported as the major component of the YOL flower. The experiment suggests that the YOL flowers have antimicrobial activity against different strains of microbes and can be used as a source of EPA (omega fatty acid) and erucamide. Currently the major amount of EPA is obtained from fish. There is the future possibility of isolation of pure EPA from YOL flower by converting the ester of EPA into EPA. Moreover,

### GC-MS analysis

The quest for GC-MS analysis was to search active alkenes and long chain fatty acids. The analysis reported the presence of the following 4 major group of compounds (Table 2)(Figure 2).

Of these 4 major compounds; Cis-5, 8, 11, 14, 17-eicosapentaenoic acid, tert-butyl dimethylsilyl ester and (Z)-docos-13-enamide or Erucamide are of great biological importance. Cis-5, 8, 11, 14, 17-eicosapentaenoic acid, tert-butyl dimethylsilyl ester is an ester derivative of eicosapentaenoic acid (EPA) which is an important long chain omega-3-fatty acid. Study on breast cancer cell lines shows that EPA in combination with Genistein proved them effective in breast cancer control.<sup>22</sup> EPA also reduces blood cholesterol level<sup>23</sup>, prevents blood platelet aggregation and protect against cardiovascular and coronary heart diseases.<sup>24</sup> Highly purified eicosapentaenoic acid prevents the progression of hepatic steatosis by repressing monounsaturated fatty acid synthesis in high-fat/high-sucrose diet-fed mice.<sup>25</sup>

On the other hand erucamide is a potent toxic compound. It is irritating to eyes, skin and respiratory system.<sup>26</sup> Synthetic erucamide is used as foam stabilizer, antiblock agent<sup>27</sup> and solvent for organic compounds. Workers in the industries using erucamide as a foam stabilizer, solvent for fats and waxes, and anti block agent for plastics are the most likely people for exposure to this compound.<sup>28</sup> The action of erucic acid on rat cardiac myoblasts in primary cell culture indicates an intense steatosis.<sup>29</sup> In another experiment, 8 of 18 rats fed with erucic acid showed myocardial granulomas, necrosis and lesions.<sup>30</sup> Erucamide is found to be readily assimilated by fungi but its biodegradation in soil and water is unknown.<sup>26</sup> However, it has been approved as an indirect food additive for use only as a component of adhesives by FDA.<sup>31</sup>

natural erucamide can replace the industrially used synthetic one. Optimization of the process involved in the extraction of the active compounds will help in scalability of production.

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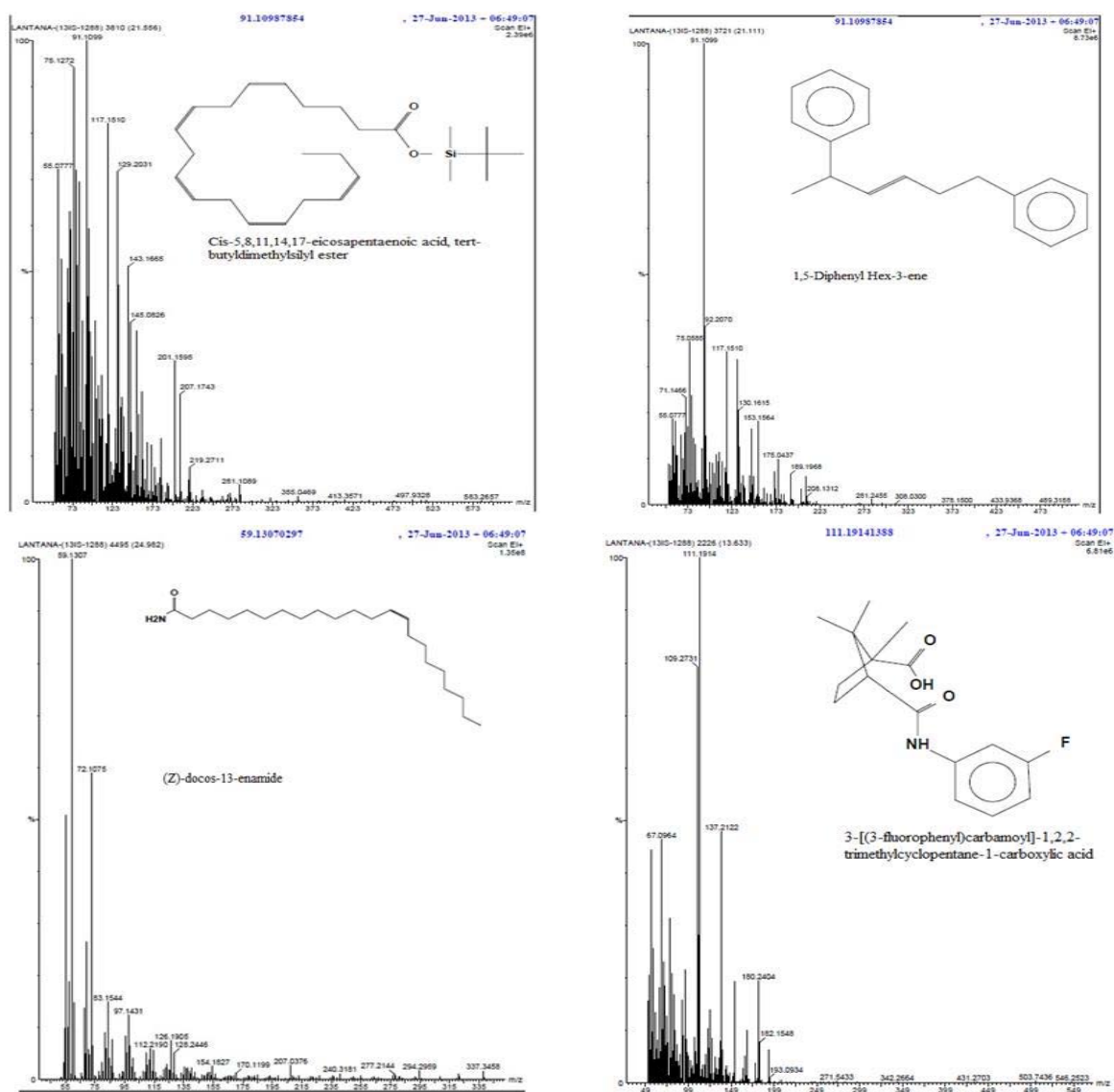


Figure 2: GC-MS peaks of the 4 major compounds in YOL flower

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