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## Antimicrobial Activity of Essential Oil Components and Antiproliferative Activity of Trans-Tiliroside Compound from an Endemic Desert Species *Thymelaea microphylla* Coss. et Dur.

Labib Noman<sup>1</sup>, Amar Zellagui<sup>1\*</sup>, Ayse Sahin Yaglioglu<sup>2</sup>, Ibrahim Demirtas<sup>2</sup> and Rhouati Salah<sup>1</sup>.

<sup>1</sup>Laboratory of Natural Products and Organic Synthesis, Department of Chemistry, Faculty of Science, University of Mentouri–Constantine, Algeria.

<sup>2</sup>Laboratory of Plant Research, Department of Chemistry, Faculty of Science, Gaziosmanpaşa University, Uluyazi Campus, Cankiri Karatekin University, Cankiri, Turkey.

### ABSTRACT

Essential oil components of the aerial parts of *Thymelaea microphylla* was subjected to evaluate the antimicrobial activity by disc diffusion method and tested against Gram-positive and Gram-negative bacteria and showed a high antibacterial activity against *E.coli* and *Staphylococcus aureus* at 2000µg /ml. The antiproliferative activity of fraction (7) from *Thymelaea microphylla* showed significant inhibitory activity on HeLa cells and moderate antiproliferative activity of trans-tiliroside compound was investigated *in vitro* on HeLa cells (human uterus carcinoma) activity and C6 cells (rat brain tumor).

**Keywords:** *T. microphylla*, Essential oil, Antimicrobial activity, Antiproliferative activity, trans-tiliroside.

\*Corresponding author

## INTRODUCTION

A natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action [1]. The use of medicinal plants as a source of medicine has been an important component of the health care system of plant extracts and bioactive compounds which isolated from medicinal plants are used for antibacterial [2]. Natural products have played an important role in cancer therapy and continue to be a promising source of new therapeutic agents [3].

*Thymelaea* Mill. genus belonging to Thymelaeaceae family. Known for its variety of secondary metabolites; diterpene esters such as tiglane type daphnane, coumarins, flavonoids, lignans, sterols, triterpene, saponins, tannins, and essential oil [4, 5] as well as their biological activities [6].

Among 31 species of *Thymelaea* Mill. genus the endemic plant *Thymelaea microphylla* Coss et Dur is one of 7 species grown in Algeria [7] has been reported to contain essential oil [5], coumarin, flavonoids, lignans [8].

The objective of this study was evaluate the antibacterial activity of essential oil and antiproliferative activity of fraction and flavonoid compound were extracted and isolated from an endemic desert species *Thymelaea microphylla* Coss. et Dur.

## MATERIAL AND METHODS

### Plant material collection

The aerial parts of *Thymelaea microphylla* Coss. et Dur. were collected in the end of March 2010 (flowering stage) in Eloued, desert of south Algeria. The plant was identified by Dr. Chahma A. M. University of Ouargla. Fresh aerial parts were dried to constant weight at room temperature. A voucher specimen was deposited at the chemistry Department University of Mentouri-Constantine under the code number ZA 107.

### Preparation of extract

Aerial parts of *Thymelaea microphylla* (2200 g) was crushed and extracted with  $\text{CH}_2\text{Cl}_2$ -MeOH (1:1) at room temperature. The extract was concentrated in vacuo to obtain a residue (103.7 g).

Essential oils were obtained by hydrodistillation of 100g of dried fruits using a Clevenger type apparatus for 3 h. diethyl ether (10 ml) was used as the collector solvent as reported in literature. After evaporation of the solvent, the oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at  $-20^\circ\text{C}$  before analysis to afford 0.02 g (0.2 %) of crude oil. The oil sample was subsequently analyzed by GC-MS [5].

### Bacterial Strains

All of the bacteria clinical strains: (*E.coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) were obtained from Bacteriology Laboratory Constantine Hospital University (C.H.U).

The antibacterial activity test was carried out on essential oil of a *Thymelaea microphylla* Coss. and Dur. using disk diffusion method [9] against four human pathogenic bacteria, including Gram positive and Gram-negative bacteria. The bacterial strains were first grown on Muller Hinton medium (MHI) at  $37^\circ\text{C}$  for 24 h prior to seeding on to the nutrient agar.

A sterile 6-mm-diameter filter disk (Whatman paper n° 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped on to each paper disk (40  $\mu\text{l}$  per disk) for all of prepared concentrations (2000 $\mu\text{g/ml}$ , 500 $\mu\text{g/ml}$ , 100 $\mu\text{g/ml}$ , 25 $\mu\text{g/ml}$ ). The treated Petri disks were kept at  $4^\circ\text{C}$  for 1 h, and incubated at  $37^\circ\text{C}$  for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate.

**Isolation and identification**

The GC analysis identified 11 compounds from *Thymelaea microphylla* Coss. et Dur. representing 100 % of the total volatile content. The major components were found to be: D-menthone (41.86 %), 2-Undecanone (23.74 %), Pulegone(11.94%) and Perillal (9.34 %). Some other compounds were only present in minor amounts. The oil composition is dominated by the Monoterpenes (67.84 %) dominated by oxygenated compounds (62.94%). Among the sesquiterpenes, oxygenated compounds represent the whole content (1.54 %) [5].

The residue of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1:1) extract (103.7 g) was fractionated by flash column chromatography eluted with hexane, followed by a gradient of hexane and CH<sub>2</sub>Cl<sub>2</sub> up to 100% CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>2</sub>Cl<sub>2</sub>-MeOH up to 100% MeOH to obtain (9) fractions. The fraction (7) (6.3 g) was separated by column chromatography to obtain (6.43 mg) pure compound.

NMR measurements were performed on a Bruker Avence III spectrometer in DMSO-d<sub>6</sub> (1H: 400 MHz; 13C: 100 MHz). Chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard. HPLC-TOF-MS spectra were recorded in the negative ion mode on an Agilent 6210 spectrometer. Column chromatography was carried out on silica gel (Merck, 60-230 mesh) in glass columns in open atmosphere pressure. For thin-layer chromatography, silica gel F254 (Merck) precoated plates were used. Compounds were detected under UV (254 nm) and sprayed with 5% seric sulphate-H<sub>2</sub>SO<sub>4</sub> reagent, followed by heating at 105 °C for 1-2 min.

**Cell proliferation assay**

Antiproliferative effects of the fraction was investigated on HeLa cell lines and the compound was investigated on HeLa and C6 cell lines using proliferation BrdU ELISA assay [10-14]. 5-Fluorouracil (5-FU) was used as positive controls.

Cultured cells were grown in 96-well plates (COSTAR, Corning, USA) at a density of 3 x 10<sup>4</sup> cells/well. In each experimental set, cells were plated in triplicates and replicated twice. The cell lines were exposed to eight concentrations of compound sample and 5-FU for 24 h at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>.

Cells were than incubated for overnight before applying the BrdU Cell proliferation ELISA assay reagent (Roche, Germany), according to manufacturer’s procedure. The amount of cell proliferation was assessed by determining the A450 nm of the culture media after addition of the substrate solution by using a microplate reader (Awareness Chromate, USA).

Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. All assays were repeated at least twice using against HeLa, and C6 cells. Percentage of inhibition of cell proliferation was calculated as follows:

$$(1- A \text{ treatments } /A \text{ vehicle control}) \times 100.$$

**RESULTS AND DISCUSSION**

**Antimicrobial study:**

Results obtained from disk diffusion method showed the antibacterial activity of plant essential oil in Table No.1.

**Table 1: Antibacterial activity of the essential oil.**

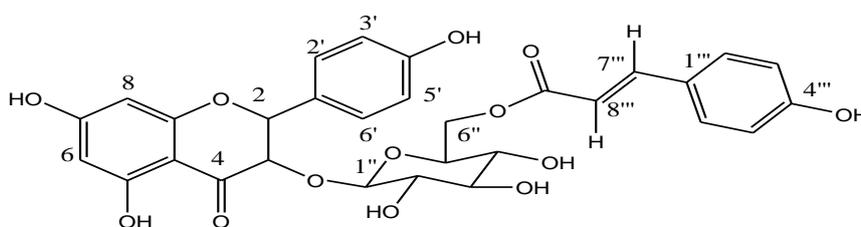
Bacterial Strains	25µg/ml	100µg/ml	500µg/ml	2000µg/ml
<i>E. coli</i>	-	18.66±01.5	25±1,73	27,33±0,57
<i>Klebsela pneumoniae</i>	9.0±0.0	10.00±0.0	23.66±2.51	27±1
<i>Pseudomonas aerogenosa</i>	-	10.66±01.15	11.33±01.15	16.00±00
<i>Staphylococcus aureus</i>	15.33±1.15	20±0.0	25.66±0.57	27.33±3.78

*In vitro* studies in this work, the antibacterial activity of essential oil of *Thymelaea microphylla* Coss. et Dur. showed significant increase in the zones of inhibition with increase in essential oil concentration and showed high antibacterial activity against Gram-negative *E.coli* and Gram-positive *Staphylococcus aureus* at 2000µg/ml concentration with inhibition zone (27,33 mm), followed by Gram-negative *Klebsiella pneumoniae* with inhibition zone (27 mm), while showed low inhibit effect against *Pseudomonas aeruginosa*.

**Identification and antiproliferative activity**

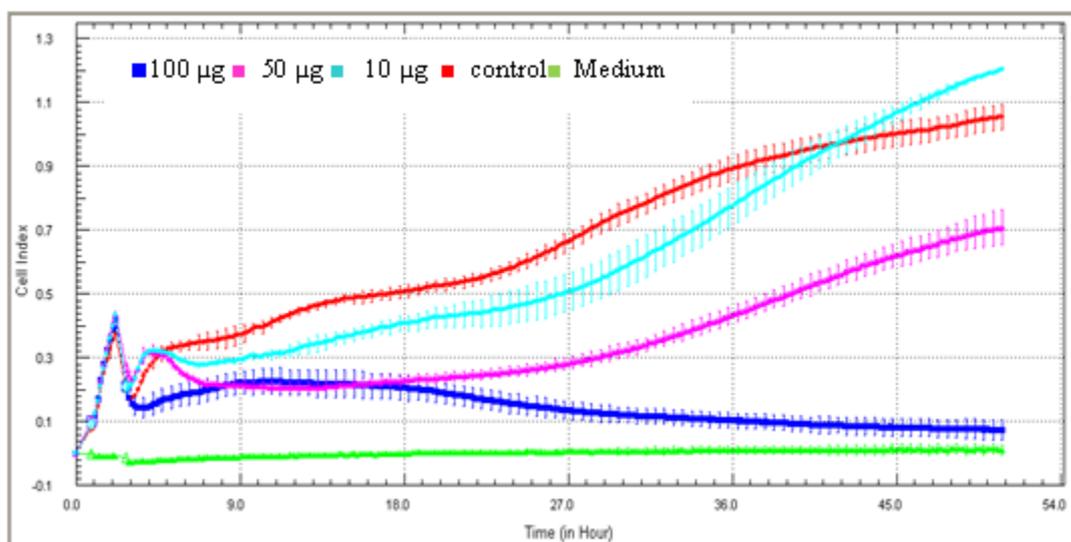
Flavonoid compound was obtained as a yellow powder analysis by HPLC-TOF/MS:  $m/z$  593.07 [M-H]<sup>-</sup>. The NMR suggests that the isolated compound is trans-tiliroside showed in figure1. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) : δ(ppm) 8.02 (2H, *d*, *J* = 9.03 Hz, H-2' & H-6'), 7.47 (1H, *d*, *J* = 15.81 Hz, H-7'''), 7.36 (2H, *d*, *J* = 8.78 Hz, H-2''' & H-6'''), 6.86 (2H, *d*, *J* = 8.78 Hz, H-3' & H-5'), 6.85 (2H, *d*, *J* = 9.3 Hz, H-3''' & H-5'''), 6.34 (1H, *d*, *J* = 2.26 Hz, H-8), 6.18 (1H, *d*, *J* = 15.81 Hz, H-8'''), 6.16 (1H, *d*, *J* = 2.26 Hz, H-6), 5.16 (1H, *d*, *J* = 7.28 Hz, H-1''), 4.34 (1H, *dd*, *J* = 11.8, 2.0 Hz, H-6''a), 4.20 (1H, *dd*, *J* = 11.81, 6.53 Hz, H-6''b), 3.50 (1H, *m*, H-3''), 3.48 (1H, *m*, H-2''), 3.47 (1H, *m*, H-5''), 3.35 (1H, *m*, H-4''), <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ(ppm) 177.32 (C, C-4), 167.49 (C, C-9'''), 161.28 (C, C-9), 160.18 (C, C-5), 160.16 (C, C-4'), 160.08 (C, C-4'''), 159.35 (C, C-7), 157.49 (C, C-2), 145.29 (CH, C-7'''), 133.69 (C, C-3), 130.70 (CH, C-2' & C-6'), 129.83 (CH, C-2''' & C-6'''), 125.58 (C, C-1'''), 121.41 (C, C-1'), 115.72 (CH, C-3''' & C-5'''), 115.51 (CH, C-3' & C-5'), 114.56 (CH, C-8'''), 113.26 (C, C-10), 103.32 (CH, C-1''), 100.59 (CH, C-6), 94.76 (CH, C-8), 76.69 (CH, C-3''), 74.41 (CH, C-2''), 74.29 (CH, C-5''), 70.24 (CH, C-4''), 62.91 (CH<sub>2</sub>, C-6'') [8].

**Figure 1: Structure of trans-tiliroside compound.**



The antiproliferative activity was investigated by using the BrdU cell proliferation ELISA assay (Roche) at several concentrations. In contrast to the 5-FU control used as a cancer drug. Fraction (7) significantly inhibited proliferation of HeLa cell lines especially at high concentrations (100 µg/ml) as shown in Fig(2).

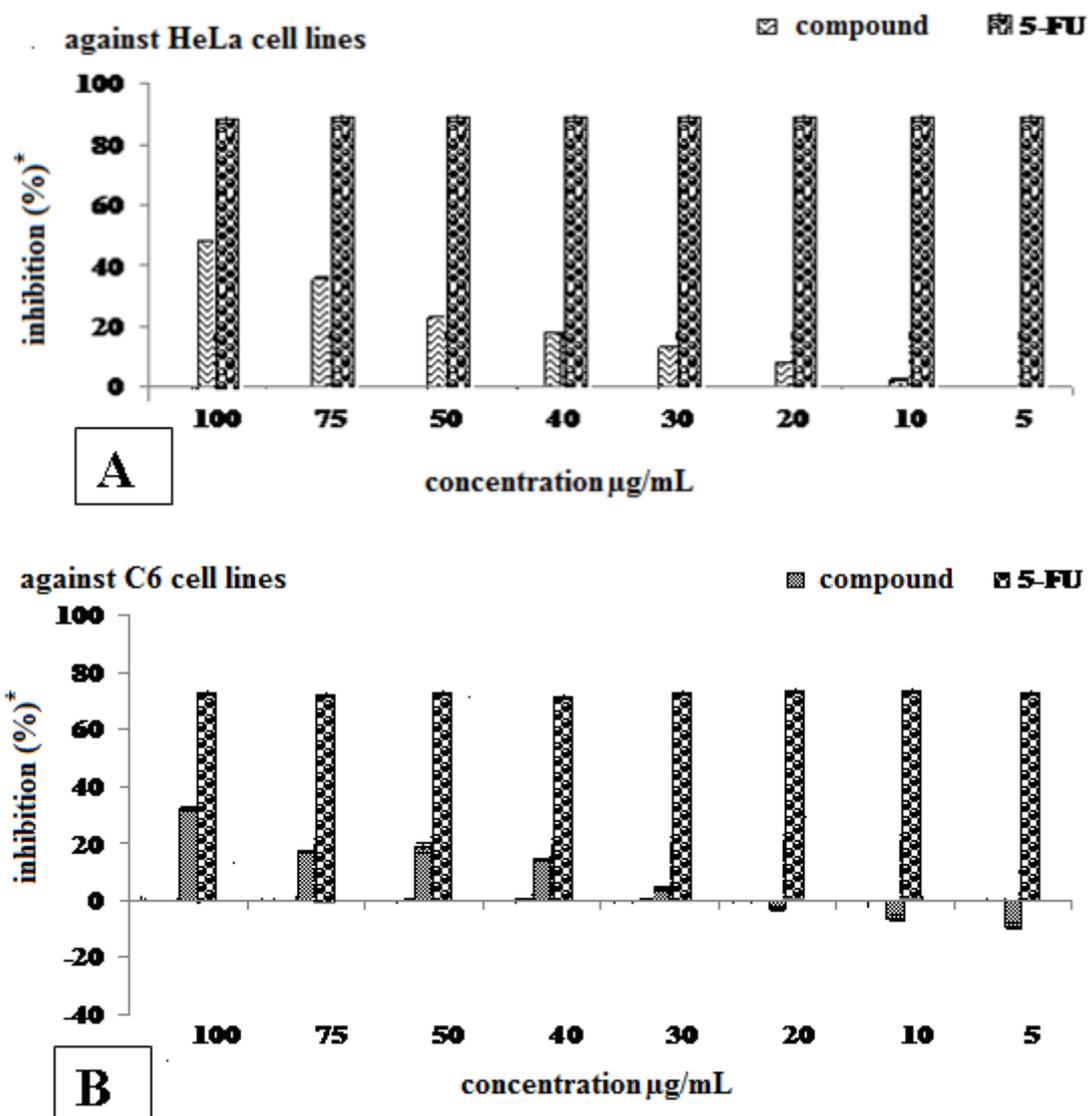
**Figure 2: Antiproliferative activity of fraction (7) against HeLa cell lines.**



According to Fig 3(A), the antiproliferative activities of flavonoid compound (trans-tiliroside) was isolated from fraction (7) was determined activity with increasing depending to concentration against HeLa cell lines and showed a moderate activity compared with 5-FU at high concentrations (100 and 75 µg/ml).

However according to Fig 3(B), the antiproliferative activities of flavonoid compound (trans-tiliroside) against C6 cell lines showed no inhibited proliferation at low concentrations (5, 10 and 20 µg/ml) and determined activity with increasing depending to concentration (30 µg/ml - 100 µg/ml), and less activity compared with trans-tiliroside antiproliferative activities of HeLa cell lines. The results showed that the extracts have the highest activity than isolated compound. Therefore, we refer this extract activity may be from synergic effects.

**Figure 3: Antiproliferative activity of the compound (trans-tiliroside) and 5-FU against HeLa (A) and C6 (B) cell lines.**  
 \*each substance was tested twice in triplicates against cell lines. Data show average of 2 individual experiments (p<0.01).



**CONCLUSION**

This study was indicated that the essential oil extracted from *Thymelaea microphylla* Coss. et Dur. possess a broad spectrum of activity against Gram-positive and Gram-negative bacteria which cause the common diseases. It was explain importance of essential oil in diseases treatment to reduce drug resistance in microorganisms and the possibility of finding new effective antimicrobial compounds.

The results of the present study of antiproliferative activities against HeLa cell lines and C6 cell lines of fraction (7) and pure compound trans-tiliroside showed the significant anti-cancer activity and lead for the future study to this plant in order to isolate and identify new active anticancer compounds.

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