**Supplementary material**

**Talaramide: a new ceramide from the cytotoxic extract of the endophytic fungus *Talaromyces wortmannii***

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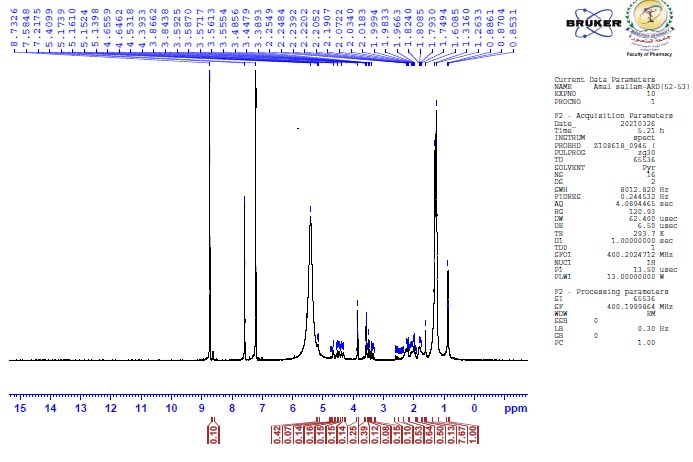
Ashraf T. Khalil: <https://orcid.org/0000-0003-1073-7375>

Amal Sallam: <https://orcid.org/0000-0001-9561-8337>

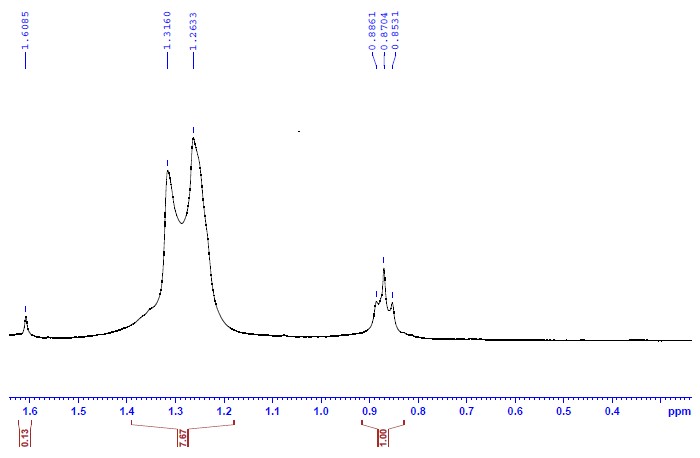
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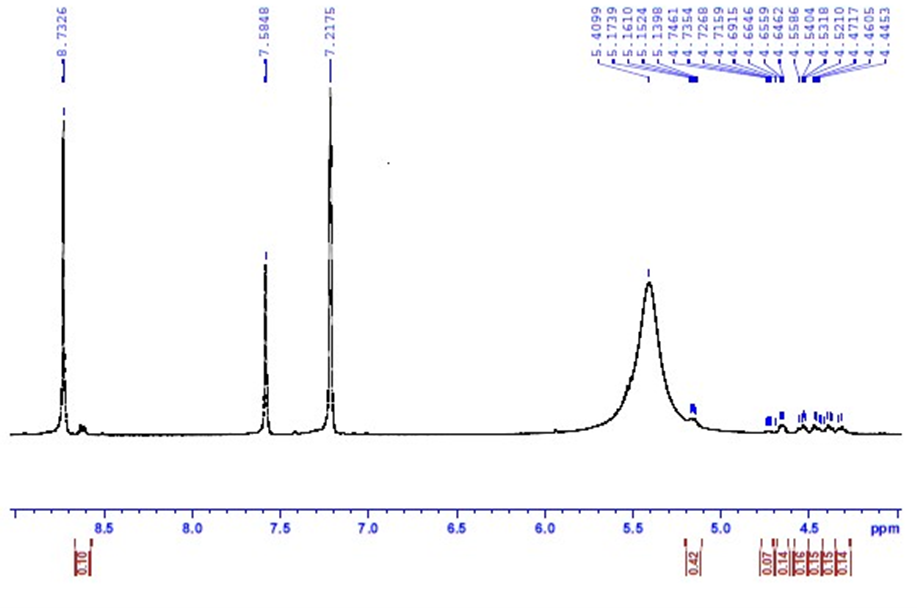
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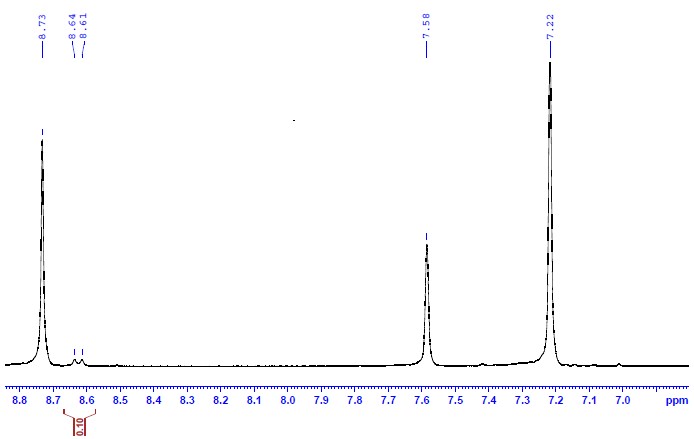
**Figure S1.** 1H NMR spectrum of compound **1A** (Pyridine-*d*5, 400 MHz).

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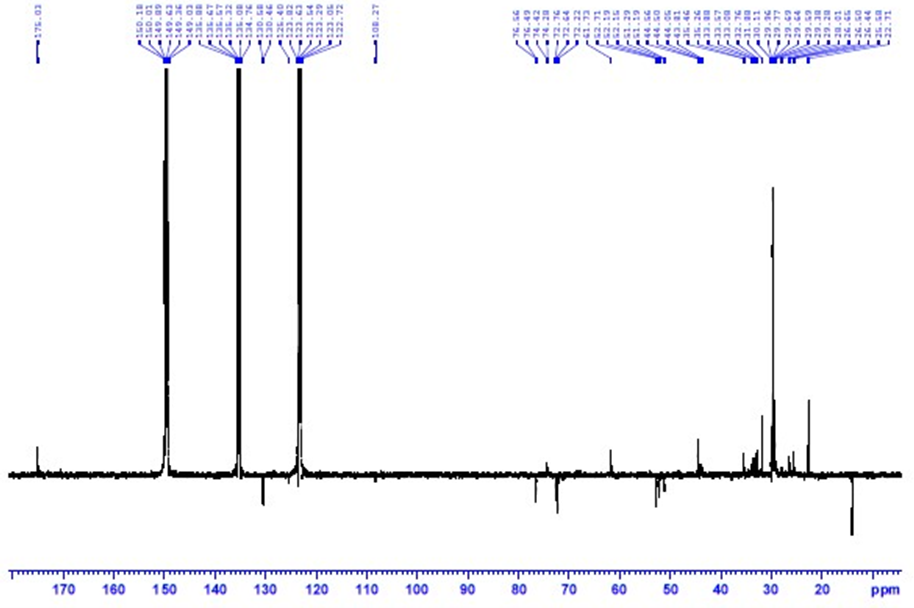
**Figure S2.** 1H NMR spectrum expansion (0.3 - 1.6 ppm) of compound **1A** (Pyridine-*d*5, 400 MHz).



**Figure S3.** 1H NMR spectrum expansion (4.0 - 9.0 ppm) of compound **1A** (Pyridine-*d*5, 400 MHz).

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**Figure S4.** 1H NMR spectrum of compound **1A** (pyridine-*d5*, 400 MHz).

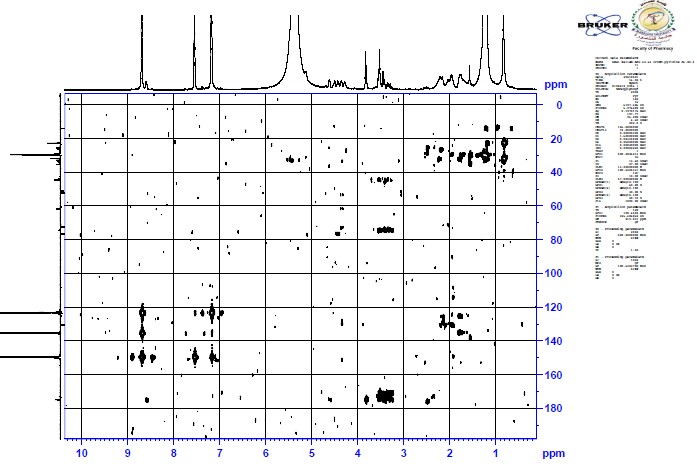
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**Figure S5.** APT spectrum of compound **1A** (Pyridine-*d*5, 100 MHz).

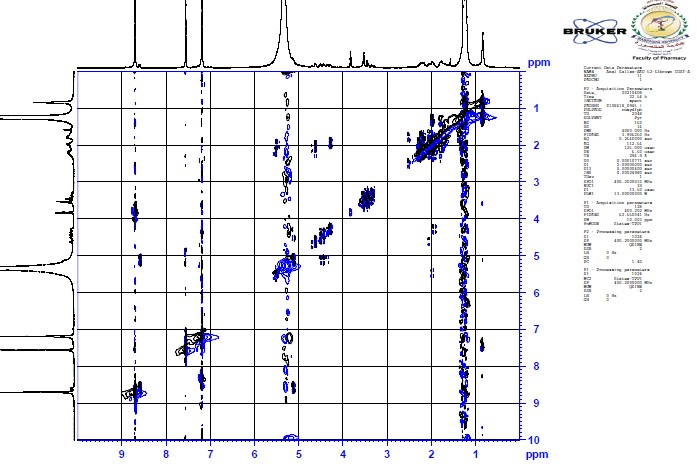
**Diagram, schematic

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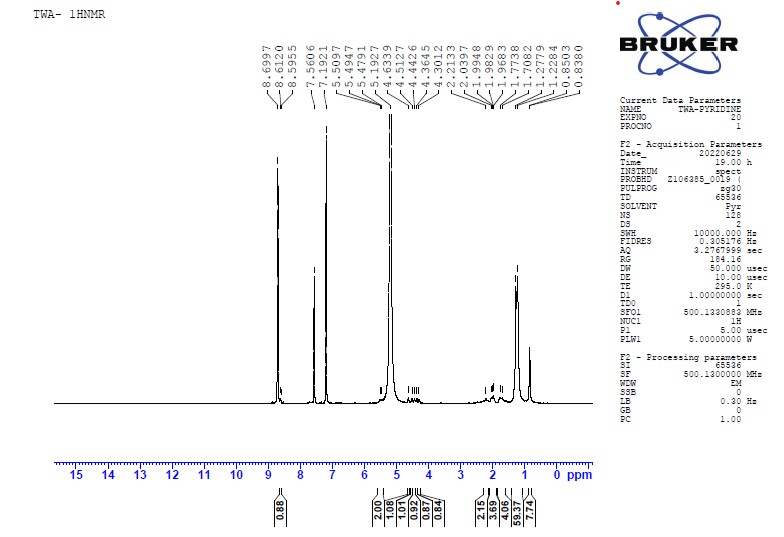
**Figure S6.** HSQC spectrum of compound **1A** (Pyridine-*d*5).

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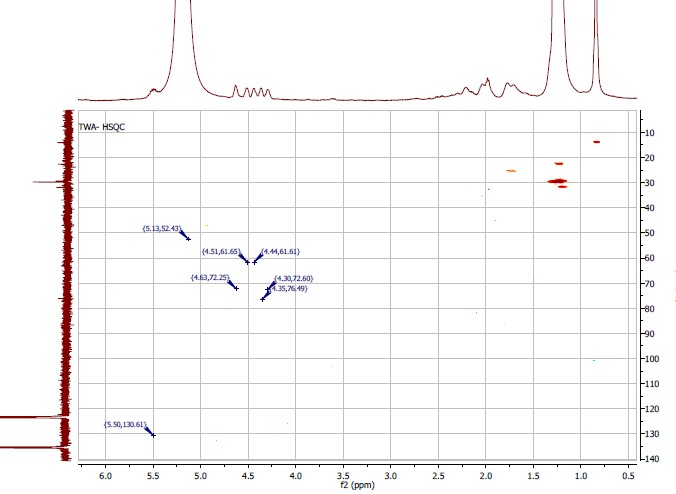
**Figure S7.** HMBC spectrum of compound **1A** (Pyridine-*d*5).

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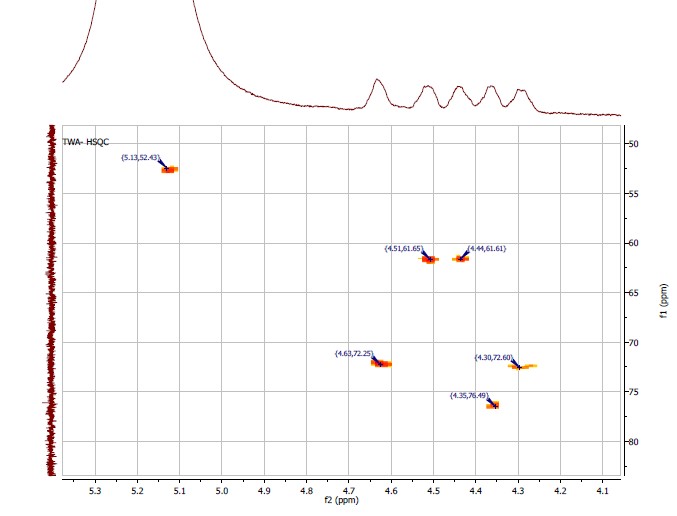
**Figure S8.** COSY spectrum of compound **1A** (Pyridine-*d*5).

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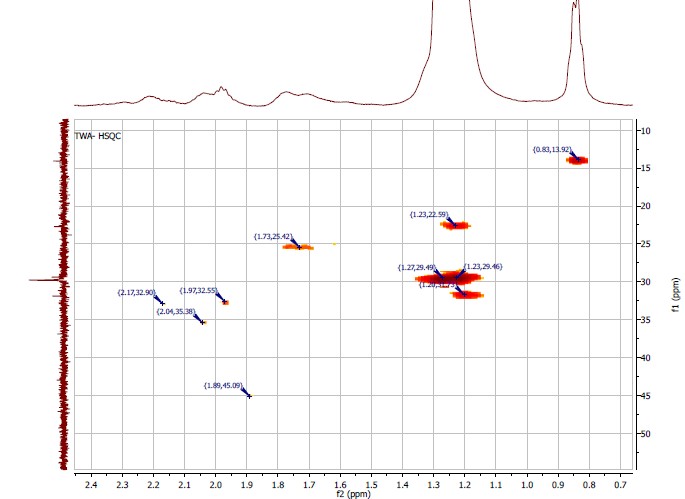
**Figure S9.** 1H NMR spectrum of compound **1** (Pyridine-*d*5, 500 MHz).

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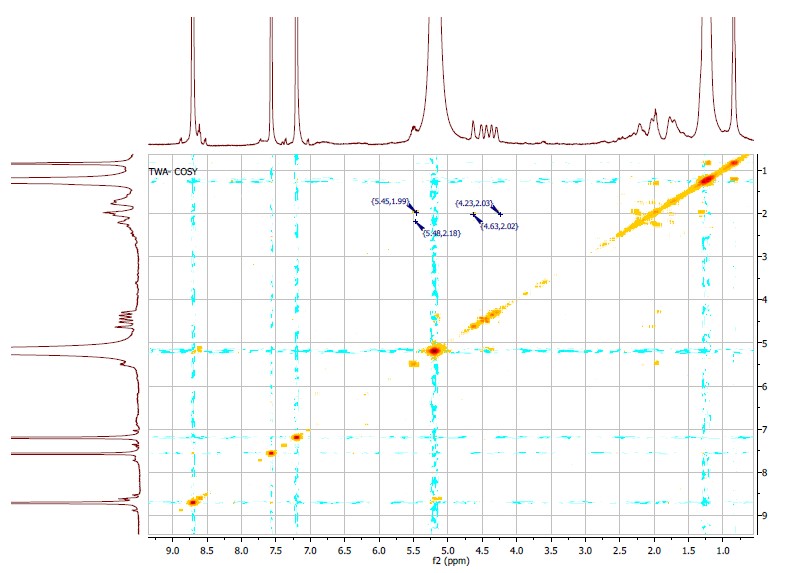
**Figure S10.** HSQC spectrum of compound **1** (Pyridine-*d*5).

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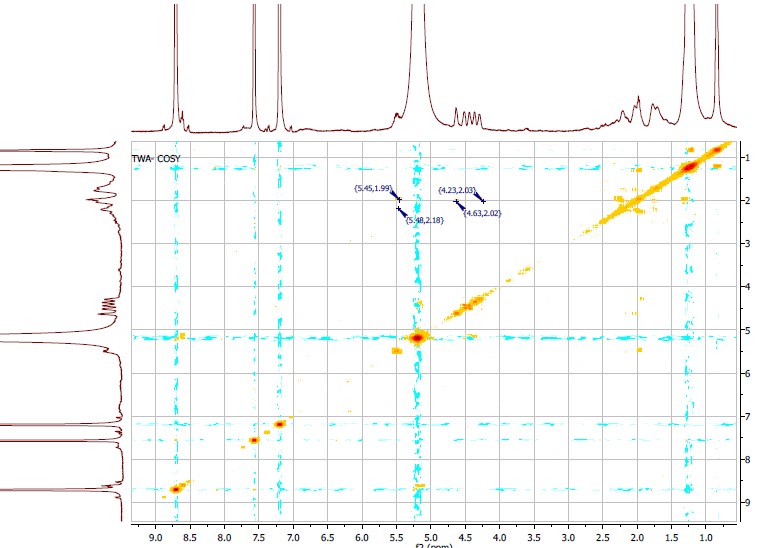
**Figure S11.** HSQC spectrum expansion of compound **1** (Pyridine-*d*5).

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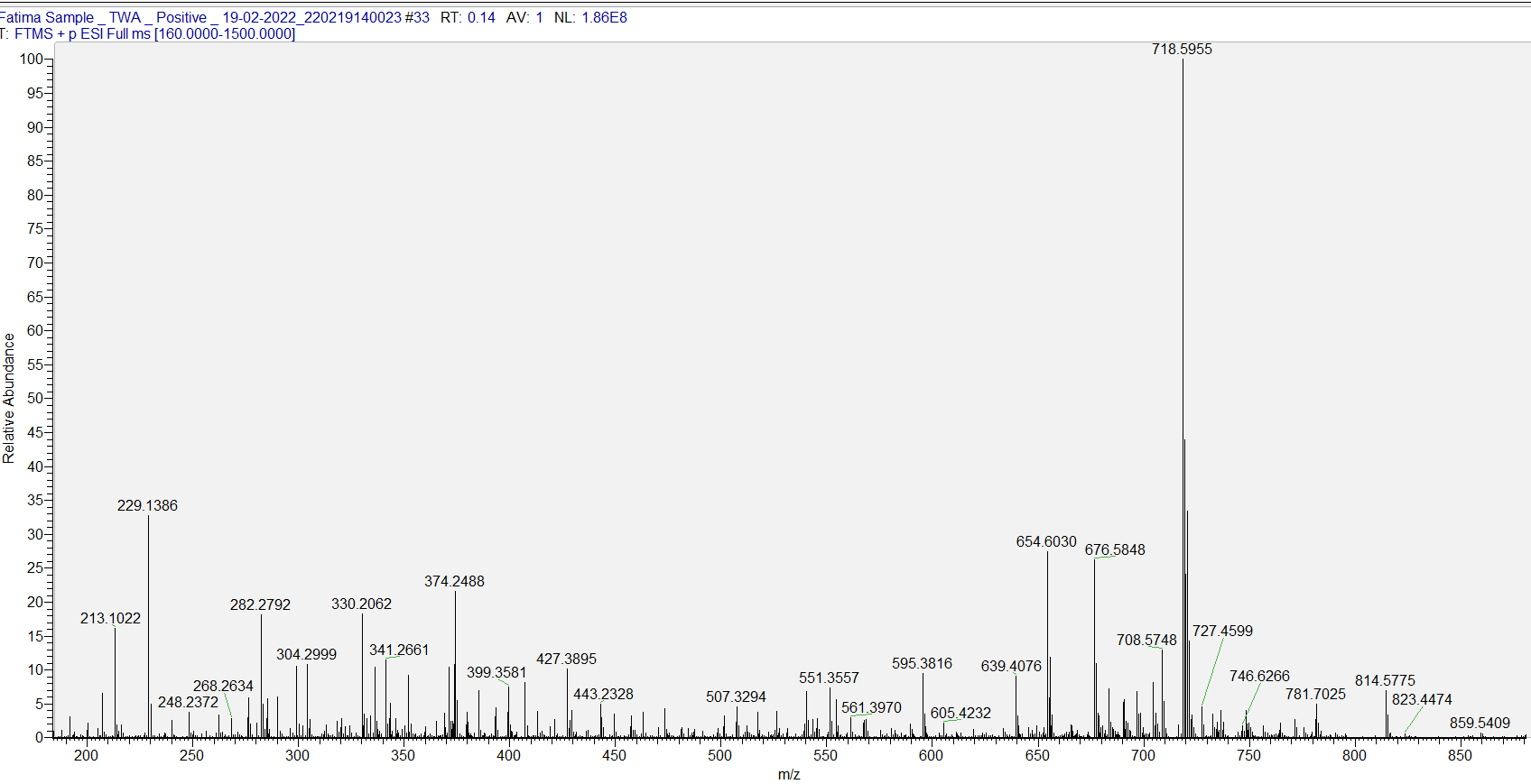
**Figure S12.** HSQC spectrum expansion of compound **1** (Pyridine-*d*5).

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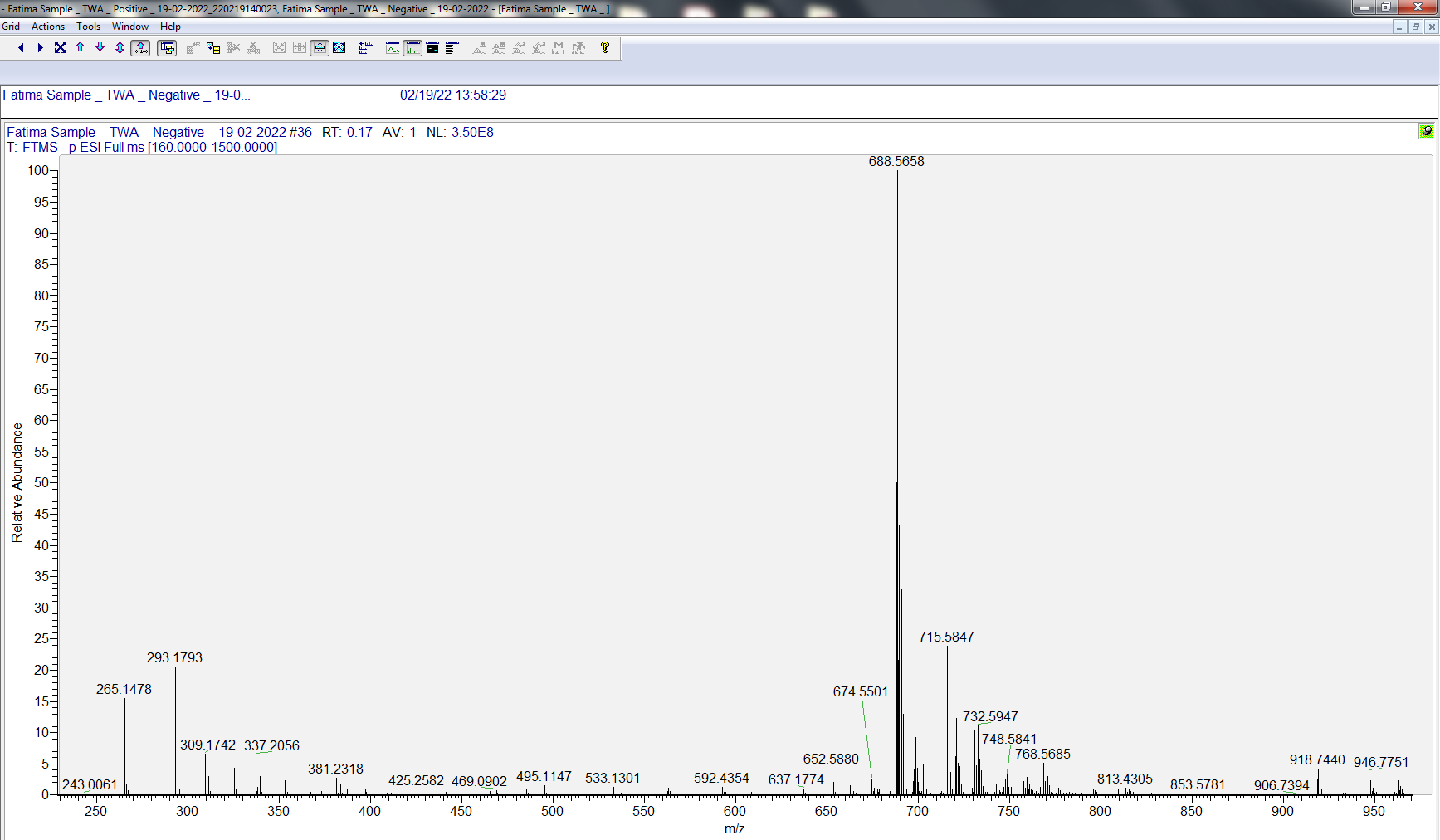
**Figure S13.** COSY spectrum of compound **1** (Pyridine-*d*5).

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**Figure S14.** NEOSY spectrum of compound **1** (Pyridine-*d*5).



**Figure S15.** HR-ESI-MS positive mode spectrum of compound **1**.



**Figure S16.** HR-ESI-MS negative mode spectrum of compound **1**.

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**Figure S17.** 1H NMR spectrum of compound **2** (Stigmasterol, CDCl3, 400 MHz).

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**Figure S18.** APT spectrum of compound **2** (Stigmasterol, CDCl3, 100 MHz).

A graph of a graph

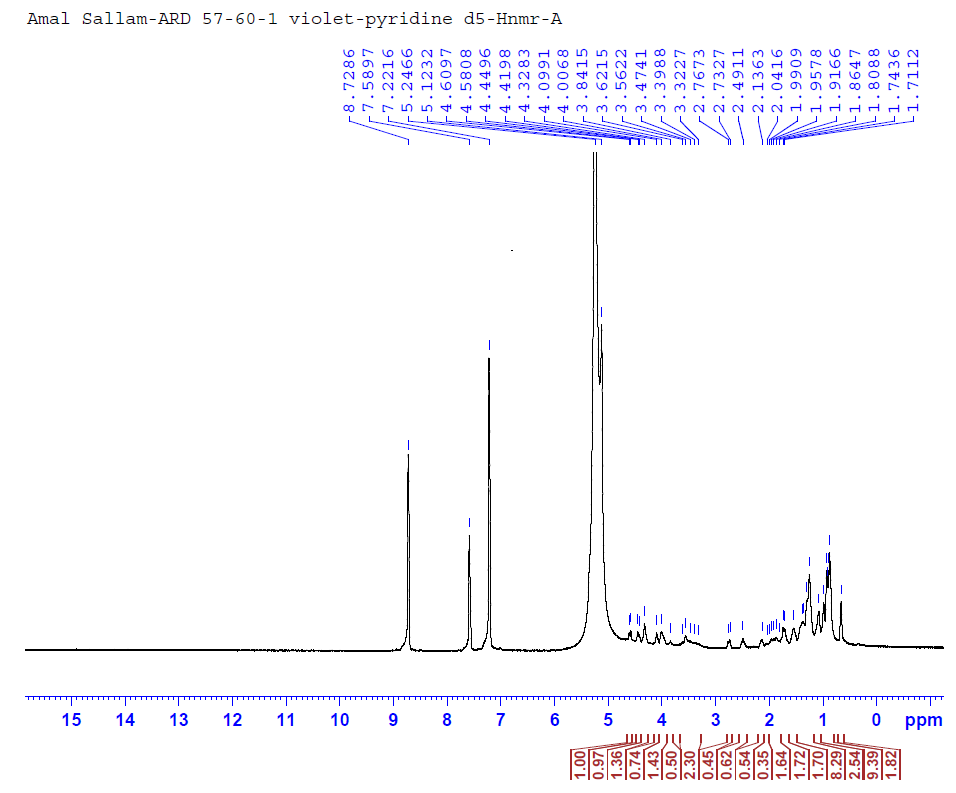
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**Figure S19.** 1H NMR spectrum of compound **3** (Thymine, pyridine-*d5*, 400 MHz).

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**Figure S20.** 1H NMR spectrum of compound **4** (Uracil, pyridine-*d5*, 400 MHz).



**Figure S21.** 1H NMR spectrum of compound **5** (Stigmasterol-3-*O*-*β*-D-glucoside pyridine-*d5*, 400 MHz).

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**Figure S22.** APT spectrum of compound **5** (Stigmasterol-3-*O*-*β*-D-glucoside pyridine-*d5*, 100 MHz).

**Table S1.** Optical densities (OD) for determination of the ethyl acetate extract of white beans culture of the endophytic fungus *Talaromyces wortmannii* isolated from *Arundo donax* L. cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on MCF7 cancer cells.

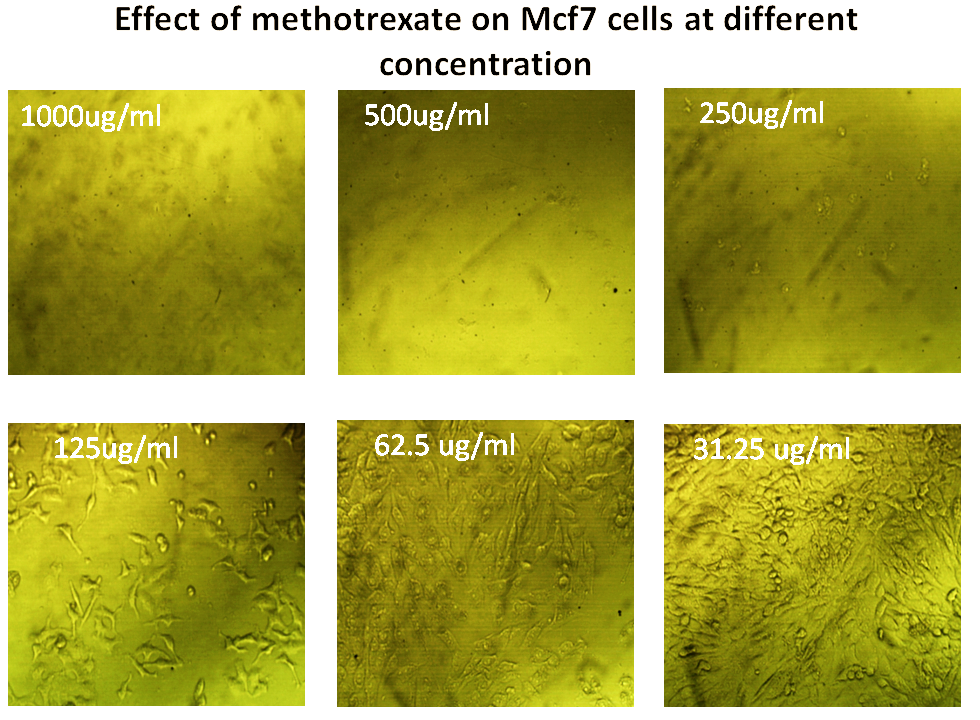
|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| Mcf7 | -------- | 0.796 | 0.808 | 0.811 | 0.805 | 0.004583 | 100 | 0 | ug |
| Talaro ext. | 1000 | 0.018 | 0.017 | 0.018 | 0.017667 | 0.000333 | 2.194616977 | 97.80538302 | 54.57 ± 0.27 |
| 500 | 0.019 | 0.02 | 0.022 | 0.020333 | 0.000882 | 2.525879917 | 97.47412008 |
| 250 | 0.043 | 0.046 | 0.06 | 0.049667 | 0.005239 | 6.169772257 | 93.83022774 |
| 125 | 0.148 | 0.154 | 0.16 | 0.154 | 0.003464 | 19.13043478 | 80.86956522 |
| 62.5 | 0.314 | 0.321 | 0.318 | 0.317667 | 0.002028 | 39.46169772 | 60.53830228 |
| 31.25 | 0.645 | 0.662 | 0.649 | 0.652 | 0.005132 | 80.99378882 | 19.00621118 |

**Table S2.** Optical densities (OD) for determination of the ethyl acetate extract of white beans culture of the endophytic fungus *Talaromyces wortmannii* isolated from *Arundo donax* L. cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on HepG2 cancer cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| HepG2 | -------- | 0.742 | 0.738 | 0.728 | 0.736 | 0.004163 | 100 | 0 | ug |
| Talaro ext. | 1000 | 0.022 | 0.021 | 0.024 | 0.022333 | 0.000882 | 3.03442029 | 96.96557971 | 57.12 ± 0.59 |
| 500 | 0.042 | 0.036 | 0.041 | 0.039667 | 0.001856 | 5.389492754 | 94.61050725 |
| 250 | 0.068 | 0.064 | 0.07 | 0.067333 | 0.001764 | 9.148550725 | 90.85144928 |
| 125 | 0.087 | 0.124 | 0.116 | 0.109 | 0.01124 | 14.80978261 | 85.19021739 |
| 62.5 | 0.296 | 0.311 | 0.303 | 0.303333 | 0.004333 | 41.21376812 | 58.78623188 |
| 31.25 | 0.675 | 0.689 | 0.672 | 0.678667 | 0.005239 | 92.21014493 | 7.789855072 |

**Table S3.** Optical densities (OD) for determination of the ethyl acetate extract of white beans culture of the endophytic fungus *Talaromyces wortmannii* isolated from *Arundo donax* L. cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on A549 cancer cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| A549 | -------- | 0.65 | 0.666 | 0.649 | 0.655 | 0.005508 | 100 | 0 | ug |
| Talaro ext. | 1000 | 0.019 | 0.02 | 0.022 | 0.020333 | 0.000882 | 3.1043257 | 96.8956743 | 84.33 ± 1.07 |
| 500 | 0.045 | 0.053 | 0.04 | 0.046 | 0.003786 | 7.022900763 | 92.97709924 |
| 250 | 0.055 | 0.063 | 0.057 | 0.058333 | 0.002404 | 8.905852417 | 91.09414758 |
| 125 | 0.163 | 0.147 | 0.15 | 0.153333 | 0.00491 | 23.40966921 | 76.59033079 |
| 62.5 | 0.364 | 0.375 | 0.352 | 0.363667 | 0.006642 | 55.5216285 | 44.4783715 |
| 31.25 | 0.661 | 0.647 | 0.651 | 0.653 | 0.004163 | 99.69465649 | 0.305343511 |

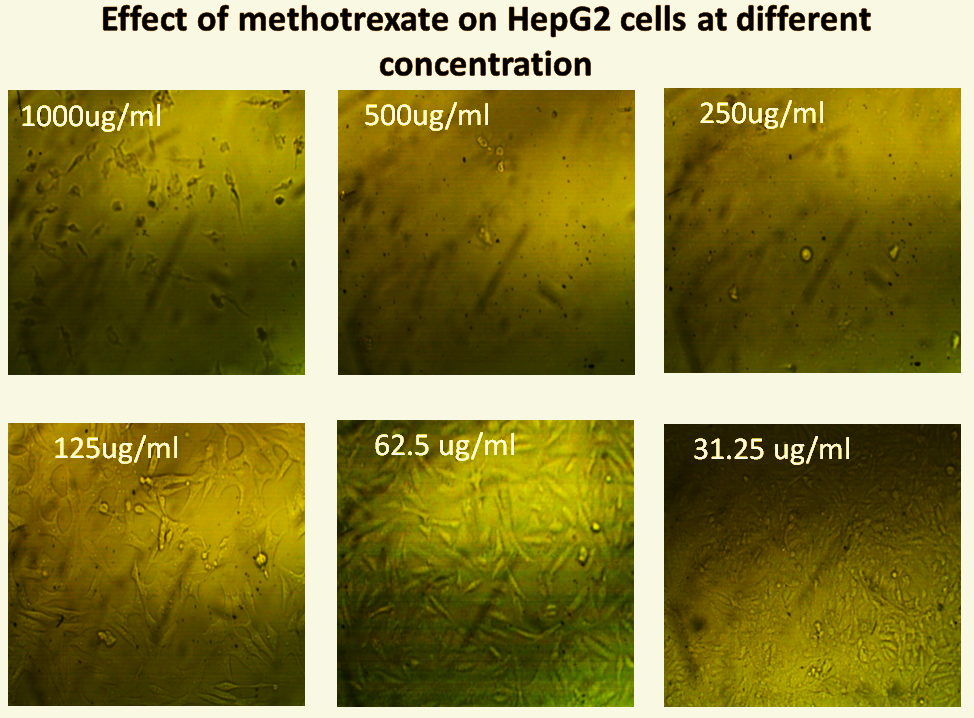


**Figure S23.** Effect of methotrexate on MCF7 cancer cells.

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**Table S4.** Optical densities (OD) for determination of methotrexate cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on MCF7 cancer cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| Mcf7 | -------- | 0.832 | 0.809 | 0.825 | 0.822 | 0.006807 | 100 | 0 | ug |
| methotrextate | 1000 | 0.019 | 0.018 | 0.019 | 0.018667 | 0.000333 | 2.270884023 | 97.72911598 | 80.25 ± 1.11 |
| 500 | 0.022 | 0.019 | 0.021 | 0.020667 | 0.000882 | 2.514193025 | 97.48580697 |
| 250 | 0.053 | 0.047 | 0.063 | 0.054333 | 0.004667 | 6.609894566 | 93.39010543 |
| 125 | 0.194 | 0.216 | 0.188 | 0.199333 | 0.008511 | 24.24979724 | 75.75020276 |
| 62.5 | 0.41 | 0.397 | 0.407 | 0.404667 | 0.00393 | 49.22952149 | 50.77047851 |
| 31.25 | 0.785 | 0.769 | 0.76 | 0.771333 | 0.007311 | 93.83617194 | 6.163828062 |

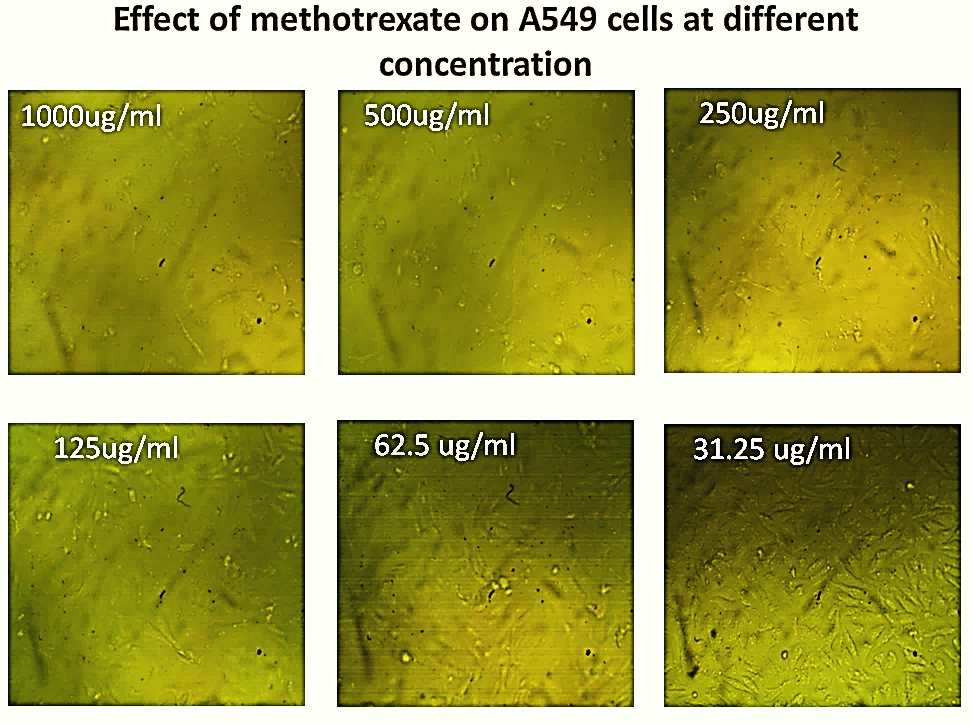


**Figure S24.** Effect of methotrexate on HepG2 cancer cells.

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**Table S5.** Optical densities (OD) for determination of methotrexate cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on HepG2 cancer cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| HepG2 | -------- | 0.732 | 0.718 | 0.725 | 0.725 | 0.004041 | 100 | 0 | ug |
| methotrextate | 1000 | 0.02 | 0.017 | 0.018 | 0.018333 | 0.000882 | 2.528735632 | 97.47126437 | 83.83 ± 0.74 |
| 500 | 0.025 | 0.053 | 0.044 | 0.040667 | 0.008253 | 5.609195402 | 94.3908046 |
| 250 | 0.089 | 0.094 | 0.088 | 0.090333 | 0.001856 | 12.45977011 | 87.54022989 |
| 125 | 0.189 | 0.179 | 0.18 | 0.182667 | 0.00318 | 25.1954023 | 74.8045977 |
| 62.5 | 0.378 | 0.385 | 0.399 | 0.387333 | 0.006173 | 53.42528736 | 46.57471264 |
| 31.25 | 0.711 | 0.695 | 0.723 | 0.709667 | 0.00811 | 97.88505747 | 2.114942529 |

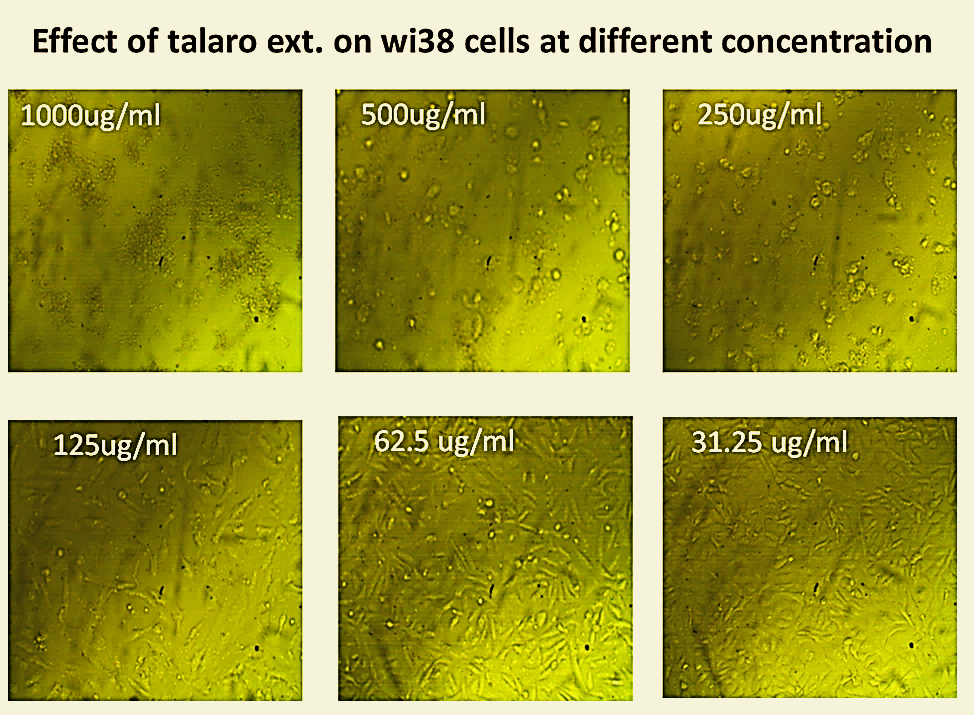


**Figure S25.** Effect of methotrexate on A549 cancer cells.

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**Table S6.** Optical densities (OD) for determination of methotrexate cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on A549 cancer cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| A549 | -------- | 0.618 | 0.633 | 0.63 | 0.627 | 0.004583 | 100 | 0 | ug |
| methotrextate | 1000 | 0.018 | 0.019 | 0.017 | 0.018 | 0.000577 | 2.870813397 | 97.1291866 | 153.2 ± 2.03 |
| 500 | 0.056 | 0.074 | 0.05 | 0.06 | 0.007211 | 9.56937799 | 90.43062201 |
| 250 | 0.097 | 0.089 | 0.104 | 0.096667 | 0.004333 | 15.41733121 | 84.58266879 |
| 125 | 0.354 | 0.34 | 0.348 | 0.347333 | 0.004055 | 55.39606592 | 44.60393408 |
| 62.5 | 0.521 | 0.538 | 0.54 | 0.533 | 0.006028 | 85.00797448 | 14.99202552 |
| 31.25 | 0.621 | 0.629 | 0.63 | 0.626667 | 0.002848 | 99.94683679 | 0.053163211 |

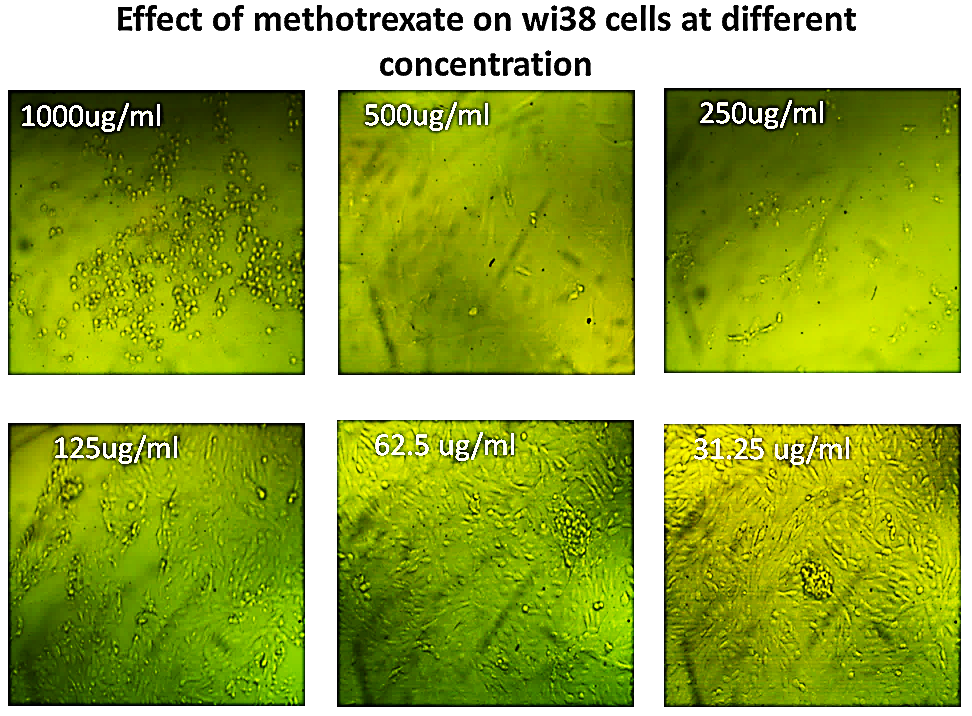


**Figure S26.** Effect of the ethyl acetate extract of white beans culture of the endophytic fungus *Talaromyces wortmannii* isolated from *Arundo donax* L. on WI38 normal cells.

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**Table S7.** Optical densities (OD) for determination of the ethyl acetate extract of white beans culture of the endophytic fungus *Talaromyces wortmannii* isolated from *Arundo donax* L. cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on WI38 normal cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| Wi38 | -------- | 0.673 | 0.678 | 0.668 | 0.673 | 0.002887 | 100 | 0 | ug |
| Talaro ext. | 1000 | 0.018 | 0.023 | 0.021 | 0.020667 | 0.001453 | 3.070827142 | 96.92917286 | 115.43 ± 1.92 |
| 500 | 0.056 | 0.063 | 0.058 | 0.059 | 0.002082 | 8.766716196 | 91.2332838 |
| 250 | 0.083 | 0.092 | 0.099 | 0.091333 | 0.004631 | 13.57107479 | 86.42892521 |
| 125 | 0.299 | 0.278 | 0.285 | 0.287333 | 0.006173 | 42.69440317 | 57.30559683 |
| 62.5 | 0.639 | 0.652 | 0.647 | 0.646 | 0.003786 | 95.98811293 | 4.011887073 |
| 31.25 | 0.675 | 0.671 | 0.67 | 0.672 | 0.001528 | 99.85141159 | 0.14858841 |



**Figure S27.** Effect of methotrexate on WI38 normal cells.

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**Table S8.** Optical densities (OD) for determination of methotrexate cytotoxicity on cells (MTT protocol) showing triplicate treatments at (1000, 500, 250, 125, 62.5, and 31.25 μg/mL) on WI38 normal cells.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **ug/ml** | **OD** | | | **Mean OD** | **±SE** | **Viability %** | **Toxicity %** | **IC50**  **± SD** |
| Wi38 | -------- | 0.685 | 0.664 | 0.673 | 0.674 | 0.006083 | 100 | 0 | ug |
| methotrextate | 1000 | 0.019 | 0.017 | 0.018 | 0.018 | 0.000577 | 2.670623145 | 97.32937685 | 156.03 ± 0.63 |
| 500 | 0.032 | 0.036 | 0.044 | 0.037333 | 0.003528 | 5.539070227 | 94.46092977 |
| 250 | 0.099 | 0.075 | 0.078 | 0.084 | 0.00755 | 12.46290801 | 87.53709199 |
| 125 | 0.365 | 0.348 | 0.35 | 0.354333 | 0.005364 | 52.57171118 | 47.42828882 |
| 62.5 | 0.664 | 0.67 | 0.673 | 0.669 | 0.002646 | 99.25816024 | 0.741839763 |
| 31.25 | 0.674 | 0.663 | 0.678 | 0.671667 | 0.004485 | 99.65380811 | 0.346191889 |

**End of Supplementary material**