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**Chemical characterization of two Egyptian *Melilotus* species to reveal their anti-inflammatory properties**

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**Supporting information**

**Table S1: Components identified by** **GC/MS of the unsaponifiable matter of *M. messanensis* and *M. indicus***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Area %** | **M.Wt** | **M.F** | **Rt** | **Compound** | **BP** | **No** |
| ***Mi*** | ***Mm*** |
| 0.7 | 1.22 | 236 | C15H24O2 | 10.83 | 2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-hydroxy-4-methyl- | 165 | **1** |
| - | 0.28 | 220 | C15H24O | 11.34 | Butylated hydroxyl toluene | 205 | **2** |
| 0.57 | 1.73 | 218 | C16H26 | 11.61 | (1-butylhexyl) Benzene | 91 | **3** |
| 0.68 | 1.62 | 218 | C16H26 | 11.72 | Benzene, (1-propylheptyl)- | 91 | **4** |
| 0.55 | 1.89 | 218 | C16H26 | 11.95 | Benzene, (1-ethyloctyl)- | 91 | **5** |
| 0.88 | 2.84 | 218 | C16H26 | 12.38 | Benzene, (1-methylnonyl)- | 105 | **6** |
| 0.80 | 2.38 | 232 | C17H28 | 12.72 | Benzene, (1-pentylhexyl)- | 91 | **7** |
| 1.99 | 6.26 | 232 | C17H28 | 12.76 | Benzene, (1-butylheptyl)- | 91 | **8** |
| 1.69 | 5.25 | 232 | C17H28 | 12.87 | Benzene, (1-propyloctyl)- | 91 | **9** |
| 2.23 | 5.99 | 232 | C17H28 | 13.12 | Benzene, (1-ethylnonyl)- | 91 | **10** |
| 2.80 | 8.69 | 232 | C17H28 | 13.54 | Benzene, (1-methyldecyl)- | 105 | **11** |
| 1.52 | 4.64 | 246 | C18H30 | 13.80 | Benzene, (1-pentylheptyl)- | 91 | **12** |
| 1.69 | 4.97 | 246 | C18H30 | 13.86 | Benzene, (1-butyloctyl)- | 91 | **13** |
| 1.29 | 4.27 | 246 | C18H30 | 13.99 | Benzene, (1-propylnonyl)- | 91 | **14** |
| 1.48 | 4.83 | 246 | C18H30 | 14.23 | Benzene, (1-ethyldecyl)- | 91 | **15** |
| 2.51 | 7.02 | 246 | C18H30 | 14.64 | Benzene, (1-methylundecyl)- | 105 | **16** |
| 1.96 | 4.88 | 260 | C19H32 | 14.85 | Benzene, (1-pentyloctyl)- | 91 | **17** |
| 1.27 | 3.33 | 260 | C19H32 | 14.92 | Benzene, (1-butylnonyl)- | 91 | **18** |
| 2.09 | - | 268 | C18H36O | 15.01 | 2-Pentadecanone, 6,10,14-trimethyl- | 43 | **19** |
| 1.62 | 3.2 | 260 | C19H32 | 15.05 | Benzene, (1-propyldecyl)- | 91 | **20** |
| 1.34 | 3.61 | 260 | C19H32 | 15.30 | Benzene, (1-ethylundecyl)- | 91 | **21** |
| 1.93 | 5.31 | 260 | C19H32 | 15.70 | Benzene, (1-methyldodecyl)- | 105 | **22** |
| 1.54 | 1.62 | 296 | C20H40O | 17.62 | Phytol | 71 | **23** |
| 4.44 | 2.78 | 281 | C18H35NO | 19.84 | 9-Octadecenamide, (Z)- | 59 | **24** |
| 1.19 | - | 326 | C22H46O | 20.78 | Behenic alcohol | 83 | **25** |
| 0.98 | - | 256 | C17H36O | 20.83 | 1-Hexadecanol, 2-methyl- | 57 | **26** |
| 5.63 | - | 380 | C27H56 | 22.33 | Heptacosane | 57 | **27** |
| - | 0.68 | 416 | C27H44O3 | 23.09 | 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3.beta.,5Z,7E)- | 207 | **28** |
| - | 1.07 | 490 | C35H70 | 23.95 | 17-Pentatriacontene | 57 | **29** |
| 36.34 | - | 298 | C20H42O | 23.99 | 1-Dodecanol, 2-octyl- | 57 | **30** |
|  | 0.91 |  | C26H44O5 | 28.31 | Ethyl iso-allocholate | 207 | **31** |
| 1.65 | - | 400 | C28H48O | 28.32 | Campesterol | 43 | **32** |
| 1.52 | 0.77 | 412 | C29H48O | 28.86 | Stigmasterol | 207 | **33** |
| 12.26 | 6.39 | 432 | C29H50O | 29.95 | gamma-Sitosterol | 207 | **34** |
| 0.48 | 0.3 | 426 | C30H50O | 30.62 | α -Amyrin | 207 | **35** |
| 1.95 | 1.21 | 426 | C30H50O | 31.57 | Lupeol | 207 | **36** |
| 98.8 | 99.9 |  | **Total identified compounds** |

BP; base peak, Rt; retention time, MF; molecular formula and M.Wt; molecular weight, Mm; *Melilotus messanensis,* Mi; *Melilotus indicus* , data represented in table according to [16,17,19,20]

**Table S2: Components identified by GC/MS of the fatty acid methyl esters of *M. messanensis* and *M.* *indicus***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Area %** | **M. Wt** | **M.F** | **Rt** | **Compound** | **BP** | **No** |
| ***Mi*** | ***Mm*** |
| - | 0.44 | 214 | C13H26O2 | 7.79 | Undecanoic acid, 10-methyl-, methyl ester | 74 | **1** |
| - | 2.63 | 242 | C15H30O2 | 8.99 | Tetradecanoic acid, methyl ester | 74 | **2** |
| - | 4.38 | 256 | C16H32O2 | 9.73 | Pentadecanoic acid, methyl ester | 74 | **3** |
| - | 1.46 | 268 | C17H32O2 | 10.57 | 9-Hexadecenoic acid, methyl ester, (Z)-  | 55 | **4** |
| 21.07 | 50.17 | 270 | C17H34O2 | 10.68 | Hexadecanoic acid, methyl ester | 74 | **5** |
| - | 1.99 | 268 | C17H32O2 | 11.30 | 2-Hexadecenoic acid, methyl ester, (E)- | 41 | **6** |
| - | 1.41 | 284 | C18H36O2 | 11.49 | Hexadecanoic acid, ethyl ester | 88 | **7** |
| - | 1.26 | 284 | C18H36O2 | 11.95 | Hexadecanoic acid, 15-methyl-, methyl ester  | 74 | **8** |
| - | 6.47 | 294 | C19H34O2 | 13.09 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester  | 67 | **9** |
| 19.40 | - | 294 | C19H34O2 | 13.10 | 9,12-Octadecadienoic acid, methyl ester, (E,E)- | 67 | **10** |
| 59.53 | 15.31 | 292 | C19H32O2 | 13.22 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-  | 79 | **11** |
| - | 10.94 | 298 | C19H38O2 | 13.69 | Octadecanoic acid, methyl ester  | 74 | **12** |
| - | 1.22 | 340 | C22H44O2 | 20.49 | Heneicosanoic acid, methyl ester  | 74 | **13** |
| - | 1.02 | 354 | C23H46O2 | 22.90 | Docosanoic acid, methyl ester  | 74 | **14** |
| - | 1.31 | 368 | C24H48O2 | 25.46 | Tricosanoic acid, methyl ester | 74 | **15** |
| 100 | 100 |  | **Total identified compounds** |

BP; base peak, Rt; retention time, MF; molecular formula and M.Wt; molecular weight, Mm; *Melilotus messanensis,* Mi; *Melilotus indicus,* data represented in table according to [16,17,19,20]

**Table S3**: **Comparative LC/ESI/MS analysis of *M. messanensis* and *M. indicus***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Peak no. (Isolate no.)** | ***Rt* (min)** | **[M-H]-** | ***m/z* fragments** | **Tentative identification** | ***Mm*** | ***Mi*** |
| 1 | 2.01 | 131 | 113, 87,70 | Asparagine | **+** | **+** |
| 2 | 2.54 | 293 | 131, 113, 87 | Asparagine-hexoside | **+** | **+** |
| 3 | 3.47 | 317 | 273, 225, 165,131 | Unknown | **+** | **-** |
| 4 | 4.48 | 191 | 129,111, 85 | Quinic acid b | **+** | **+** |
| 5 | 8.28 | 371 | 195,99, 80 | Unknown | **+** | **-** |
| 6 | 14.82 | 329 | 167,153, 109 | Vanillic acid-*O*-glucoside b,c | **+** | **-** |
| 7(**21**) | 16.15 | 327 | 193, 176, 147, 133, 109 | Melimessanol B a,b | **+** | **+** |
| 8 | 20.43 | 337 | 191, 173, 163, 119 | Coumaroyl quinic acid | **+** | **-** |
| 9(**3**) | 20.82 | 353 | 191, 179, 161, 109 | Chlorogenic acid a,b | **+** | **-** |
| 10(**1**) | 21.7 | 136.9 | 92.9 | *p*-Hydroxy benzoic acid a | **+** | **-** |
| 11(**2**) | 22.91 | 325 | 163, 119 | *trans* *p*-Coumaric acid-*O*-glucoside a,b | **+** | **+** |
| 12 | 25.23 | 801 | 639, 477, 315 | Isorhamnetin *O*-dihexoside-*O*-hexoside | **+** | **-** |
| 13 | 25.36 | 771 | 609, 463, 301 | Quercetin 3-*O*-(rhamnosyl hexoside)-7-*O*-hexoside | **-** | **+** |
| 14 | 27.2 | 755 | 593, 447, 285 | Kaempferol 3-*O*-(rhamnosyl hexoside)-7-*O*-hexoside | **-** | **+** |
| 15(**10**) | 28.4 | 885 | 739,593, 431, 285 | Kaempferol 3-*O*-(2՛՛,6՛՛ di α-rhamno-pyranosyl)-*O*-β-galactopyranoside-7-*O*-α-rhamnopyranoside a,b | **+** | **+** |
| 16 | 30.97 | 325 | 163, 119 | Coumaroyl glucoside isomer | **+** | **+** |
| 17(**15**) | 32.18 | 755 | 609, 447, 301 | Quercetin3-*O*-(6՛՛-α-rhamnopyranosyl)-*O*-β-galactopyranoside-7-*O*-α-rhamno-pyranoside a,b | **+** | **+** |
| 18(**19**) | 32.84 | 447 | 429,357,327 | Isoorientin a,b | **+** | **-** |
| 19(**20**) | 33.25 | 447 | 357,327 | Orientin a,b | **+** | **-** |
| 20(**9**) | 34.8 | 739 | 593, 285 | Kaempferol3-*O*-(2՛՛,6՛՛ di α-rhamno-pyranosyl)-*O*-β-galactopyranoside a,b | **+** | **+** |
| 21(**18**) | 36.18 | 769 | 315, 314 | Isorhamnetin 3-*O*-(2՛՛,6՛՛ di α-rhamno-pyranosyl)-*O*-β-galactopyranoside a,b | **+** | **+** |
| 22(**14**) | 36.18 | 609 | 301, 300 | Quercetin 3-*O*-rutinoside a,b  | **+** | **-** |
| 23(**13**) | 37.25 | 463 | 301, 300 | Quercetin3-*O*-glucoside a,b,c  | **+** | **+** |
| 24 | 38.85 | 927 | 781, 473, 431, 430, 285 | Kaempferol 3-*O*-(di-rhamnosyl)-*O*-hexoside-7-*O*-acetyl-rhamnoside | **+** | **+** |
| 25 | 39.38 | 461 | 285 | Kaempferol 7-*O*-glucuronide b | **+** | **-** |
| 26(**8**) | 39.95 | 593 | 285 | Kaempferol3-*O*-rutinoside a,b | **-** | **+** |
| 27(**12**) | 40.7 | 447 | 301, 285 | Quercetin 3-*O*-rhamnoside a,b | **+** | **-** |
| 28(**17**) | 41.25 | 623 | 315 | Isorhamnetin 3-*O*-rutinoside a,b,c | **+** | **+** |
| 29(**7**) | 42.08 | 447 | 285, 284 | Kaempferol3-*O*-glucoside a,b | **-** | **+** |
| 30(**16**) | 42.45 | 477 | 315, 314 | Isorhamnetin 3-*O*-glucoside a,b,c | **-** | **+** |
| 31(**5**) | 42.99 | 431 | 285 | Kaempferol7-*O*-rhamnoside a,b | **+** | **+** |
| 32 | 43.79 | 445 | 269, 225, 171, 151, 117 | Medicarpin-*O*-glucuronide | **+** | **-** |
| 33(**6**) | 44.17 | 431 | 285, 284 | Kaempferol3-*O*-rhamnoside a,b | **+** | **+** |
| 34 | 44.46 | 295 | 281, 267, 167, 145, 117 | Coumesterol dimethyl ether c | **+** | **-** |
| 35 | 45.52 | 447 | 285 | Kaempferol 7-*O*-glucoside b | **+** |  |
| 36(**4**) | 45.9 | 285 | 151, 145, 117 | Kaempferol a,b,c | **-** | **+** |
| 37(**11**) | 48.2 | 301 | 179, 151,121 | Quercetin a,b,c | **-** | **+** |
| 38 | 48.79 | 267 | 167, 145, 117 | Coumesterol c | **+** | **-** |
| 39 | 53.81 | 583 | 463, 436, 343, 316 | Peptide derivative  | **+** | **-** |
| 40 | 56.48 | 269 | 225, 171, 151, 117 | Medicarpin c | **+** | **-** |
| 41 | 60.75 | 753 | 281, 253, 245, 227, 153 | Acylphosphatidylglycerol(18:1/16:1) derivative  | **+** | **-** |

a: Compounds isolated and identified in the present study, b: Compounds identified by comparing their retention times and mass spectrum with the authentic, c: Compounds isolated previously from *Melilotus messanensis* and *Melilotus indicus*; Mm: *M. messanensis*; Mi: *M. indicus,* data represented in table according [16,17,19,20]

**Figure S1.** Isolated compounds of *Melilotus indicus* and *Melilotus messanensis* [16,17,19]