**Supplementary data file**

**Antioxidant, Anti-inflammatory, Anti-nociceptive, Tyrosinase inhibitory potential and Toxicological evaluation of *Portulacaria afra* using in-vitro, In-*vivo* and *In-silico* studies**

**List of Tables**

**Table 1S.** Compounds tentatively identified in RP-UHPLC-MS (Positive mode) analysis of PAME.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound #** | **RT** | **Molecular mass** | **Identification** | **Molecular****Formula** | **MFG****Diff ppm** | **DB Diff****ppm** | **Hits** |
| 1 | 0.65 | 152.0672 |  | C5 H12 O5 | 8.22 |  |  |
| 2 | 0.651 | 189.1224 | NG-amino-L-Arginine | C6 H15 N5 O2 | 0.73 | 0.73 | 1 |
| 3 | 0.652 | 197.0894 | - | C6 H15 N O6 | 2.9 |  |  |
| 4 | 0.68 | 158.044 | - | C4 H6 N4 O3 | 0.2 |  |  |
| 5 | 0.682 | 192.0619 | BEC |  |  | -1.48 | 1 |
| 6 | 9.644 | 170.0952 | Furfural diethyl acetal | C9 H14 O3 | -5.11 | -5.11 | 3 |
| 7 | 10.166 | 183.107 | - | C10 H17 N S | 6.13 |  |  |
| 8 | 10.169 | 200.1231 | - | C11 H20 O S | 2.07 |  |  |
| 9 | 11.497 | 226.1564 | Dihydrojasmonic Acid, Methyl Ester | C13 H22 O3 | 2.18 | 2.18 | 3 |
| 10 | 11.711 | 238.1206 | - | C13 H18 O4 | -0.32 | -0.32 | 3 |
| 11 | 11.716 | 162.0671 | - | C10 H10 O2 | 6.27 |  |  |
| 12 | 11.882 | 162.0674 | - | C10 H10 O2 | 3.89 | 3.89 | 10 |
| 13 | 11.883 | 238.1203 | 2,2,4,4,-Tetramethyl-6-(1-oxopropyl)-1,3,5-cyclohexanetrione | C13 H18 O4 | 0.77 | 0.77 | 3 |
| 14 | 12.173 | 273.2661 | C16 Sphinganine | C16 H35 N O2 | 2.62 | 2.62 | 10 |
| 15 | 12.225 | 229.2389 | - | C14 H31 N O | 7.23 |  |  |
| 16 | 12.25 | 317.291 | - | C14 H35 N7 O | -2.26 |  |  |
| 17 | 12.474 | 239.1515 | Albuterol | C13 H21 N O3 | 2.52 | 2.51 | 10 |
| 18 | 12.478 | 227.1875 | - | C13 H25 N O2 | 4.53 |  |  |
| 19 | 12.485 | 199.2289 | - | C13 H29 N | 5.59 |  |  |
| 20 | 12.53 | 256.106 | 5-Ethyl-5-(1-methyl-3-carboxypropyl) barbituric acid | C11 H16 N2 O5 | -0.2 | -0.2 | 6 |
| 21 | 12.56 | 157.1461 | - | C9 H19 N O | 3.72 |  |  |
| 22 | 12.74 | 213.244 | - | C14 H31 N | 7.78 |  |  |
| 23 | 12.817 | 254.1513 | - | C14 H22 O4 | 1.98 | 1.98 | 2 |
| 24 | 12.917 | 267.1468 | - | C14 H21 N O4 | 0.89 | 0.89 | 10 |
| 25 | 13.406 | 452.3291 | - | C30 H44 O3 | -0.17 | -0.17 | 9 |
| 26 | 13.408 | 488.3505 | Arjunolic acid | C30 H48 O5 | -0.66 | -0.66 | 10 |
| 27 | 13.412 | 176.047 | 4-Methylumbelliferone | C10 H8 O3 | 2.05 | 2.05 | 10 |
| 28 | 13.413 | 222.092 | Diglycidyl resorcinol ether | C12 H14 O4 | -12.42 | -12.42 | 10 |
| 29 | 13.94 | 285.1371 | Hydromorphone | C17 H19 N O3 | -1.98 | -1.99 | 10 |
| 30 | 14.118 | 414.2049 | Eplerenone | C24 H30 O6 | -1.66 | -1.66 | 10 |
| 31 | 14.457 | 198.1606 | - | C12 H22 O2 | 6.9 |  |  |
| 32 | 14.463 | 110.1082 | - | C8 H14 | 12.12 |  |  |
| 33 | 14.658 | 194.095 | Ethyl 4-methyl-phenoxy acetate | C11 H14 O3 | -3.86 | -3.86 | 10 |
| 34 | 15.326 | 195.1427 | - |  |  |  |  |
| 35 | 15.386 | 292.1685 | 9-Acetoxyfukinanolide | C17 H24 O4 | -3.46 | -3.46 | 8 |
| 36 | 15.744 | 220.1472 | - | C14 H20 O2 | -4.04 |  |  |
| 37 | 15.883 | 256.241 | 2-hexyl-decanoic acid | C16 H32 O2 | -2.94 | -2.94 | 10 |
| 38 | 16.034 | 212.1786 | 7Z-Undecenyl acetate | C13 H24 O2 | -4.52 | -4.52 | 10 |
| 39 | 16.038 | 248.1768 | C16:4n-0,4,8,12 | C16 H24 O2 | 3.48 | 3.48 | 10 |
| 40 | 16.113 | 162.0667 | - | C10 H10 O2 | 8.54 |  |  |
| 41 | 16.114 | 342.1461 | Deoxymiroestrol | C20 H22 O5 | 1.81 | 1.81 | 7 |
| 42 | 16.437 | 162.067 | - | C10 H10 O2 | 6.67 |  |  |
| 43 | 16.438 | 342.1467 | Deoxymiroestrol | C20 H22 O5 | 0.12 | 0.12 | 7 |
| 44 | 16.497 | 294.2173 | - | C14 H26 N6 O | -1.67 |  |  |
| 45 | 16.55 | 342.1467 | Deoxymiroestrol | C20 H22 O5 | 0.12 | 0.12 | 7 |
| 46 | 16.555 | 162.0666 | - | C10 H10 O2 | 9.35 |  |  |
| 47 | 16.68 | 294.2173 | - | C14 H26 N6 O | -1.77 |  |  |
| 48 | 16.982 | 278.1514 | Emmotin A | C16 H22 O4 | 1.34 | 1.34 | 7 |
| 49 | 16.987 | 148.0152 | - | C8 H4 O3 | 5.65 |  |  |
| 50 | 17.003 | 204.0779 | 3-Butylidene-7-hydroxyphthalide | C12 H12 O3 | 3.83 | 3.82 | 4 |
| 51 | 17.478 | 229.2056 | 2S-amino-tridecanoic acid | C13 H27 N O2 | -6.01 | -6.01 | 6 |
| 52 | 17.988 | 467.3821 |  | C21 H45 N11 O | -2.58 |  |  |
| 53 | 18.011 | 402.2262 | Acetyl tributyl citrate | C20 H34 O8 | -2.14 | -2.15 | 6 |
| 54 | 18.047 | 286.214 | 2,3-Dihydroxycyclopentaneundecanoic acid | C16 H30 O4 | 1.33 | 1.33 | 5 |
| 55 | 18.048 | 110.1085 |  | C8 H14 | 9.27 |  |  |
| 56 | 18.049 | 198.1617 | 4Z-Decenyl acetate | C12 H22 O2 | 1.27 | 1.27 | 10 |
| 57 | 18.068 | 406.3303 |  - | C16 H42 N10 S | 2.94 |  |  |
| 58 | 18.142 | 362.3032 | - | C20 H42 O5 | 0.14 |  |  |
| 59 | 18.2 | 318.2788 |  - | C19 H34 N4 | -1.42 |  |  |
| 60 | 18.256 | 274.25 | - | C16 H34 O3 | 2.83 |  |  |
| 61 | 18.502 | 569.4499 | - | C26 H55 N11 O3 | -1.78 |  |  |
| 62 | 18.587 | 525.4233 | - | C24 H51 N11 O2 | -1.18 |  |  |
| 63 | 18.674 | 481.3969 | - | C22 H47 N11 O | -0.91 |  |  |
| 64 | 18.746 | 399.414 | Cerotic acid(d3) |  |  | 3.9 | 1 |
| 65 | 18.94 | 332.2917 | - | C19 H40 O4 | 2.82 |  |  |
| 66 | 19.003 | 288.2648 | - | C13 H32 N6 O | -3.63 |  |  |
| 67 | 19.006 | 106.0626 | - | C4 H10 O3 | 3.94 |  |  |
| 68 | 19.233 | 288.2652 | - | C17 H36 O3 | 4.34 |  |  |
| 69 | 19.383 | 255.2544 | - | C16 H33 N O | 7.1 |  |  |
| 70 | 19.482 | 227.2229 | - | C14 H29 N O | 8.77 |  |  |
| 71 | 19.517 | 330.2754 | 1-Monopalmitin | C19 H38 O4 | 4.84 | 4.84 | 4 |
| 72 | 19.519 | 694.6054 | - |  |  |  |  |
| 73 | 19.7 | 227.2238 | - | C14 H29 N O | 5.08 |  |  |
| 74 | 19.721 | 407.3598 | - | C18 H45 N7 O3 | -3.52 |  |  |
| 75 | 19.762 | 474.2897 | - | C30 H38 N2 O3 | -3.06 |  |  |
| 76 | 19.846 | 302.2832 | - | C18 H38 O3 | -3.71 |  |  |
| 77 | 19.95 | 353.3289 | Anandamide (20:l, n-9) | C22 H43 N O2 | 1.36 | 1.36 | 1 |
| 78 | 20.115 | 302.28 |  | C14 H34 N6 O | -1.94 |  |  |
| 79 | 20.203 | 224.2134 | 3,7,11-Trimethyl-6E,10-dodecadien-1-ol | C15 H28O | 2.72 | 2.72 | 10 |
| 80 | 20.219 | 208.1454 | (5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol | C13 H20 O2 | 4.23 | 4.23 | 10 |
| 81 | 20.307 | 333.3013 | 2,4,12-Octadecatrienoic acid isobutylamide | C22 H39 N O | 5.5 | 5.5 | 2 |
| 82 | 20.32 | 394.2161 | - | C17 H35 Cl N4 O2 S | 1.97 |  | 10 |
| 83 | 20.429 | 224.2135 | 3,7,11-Trimethyl-6E,10-dodecadien-1-ol | C15 H28 O | 2.38 | 2.39 | 10 |
| 84 | 20.464 | 208.1461 | (5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol | C13 H20 O2 | 0.95 | 0.95 | 10 |
| 85 | 20.536 | 110.0134 | - | C9 H2 | 20.76 |  |  |
| 86 | 20.68 | 383.3348 | - | C19 H41 N7 O | 6.42 |  | 1 |
| 87 | 20.738 | 627.1869 | - | C35 H37 N3 S4 | 0.23 |  |  |
| 88 | 20.761 | 622.2808 | - | C37 H34 N8 O2 | -0.48 |  |  |
| 89 | 20.843 | 255.2566 | Palmitic amide | C16 H33 N O | -1.39 | -1.39 | 10 |
| 90 | 21.091 | 255.256 | Palmitic amide | C16 H33 N O | 0.71 | 0.71 | 10 |
| 91 | 21.327 | 390.2806 | - | C21 H42 O4 S | -0.5 |  |  |
| 92 | 21.42 | 309.3025 | N-Hexadecanoylpyrrolidine | C20 H39 N O | 2.19 | 2.19 | 8 |
| 93 | 21.435 | 370.3076 | Docosanedioic acid | C22 H42 O4 | 1.8 | 1.8 | 3 |
| 94 | 21.458 | 283.2875 | Stearamide | C18 H37 N O | 0.21 | 0.21 | 10 |
| 95 | 21.636 | 663.245 | - | C46 H34 Cl N3 | -1.26 |  | 3 |
| 96 | 21.832 | 269.2708 | Capsi-amide | C17 H35 N O | 3.81 | 3.81 | 10 |
| 97 | 22.203 | 283.286 | - | C18 H37 N O | 5.37 |  |  |
| 98 | 22.736 | 283.2869 | Stearamide | C18 H37 N O | 2.22 | 2.22 | 10 |
| 99 | 23.976 | 609.1755 | - | C37 H28 Cl N5 S | -0.24 |  |  |
| 100 | 24.125 | 426.3714 | Hexacosanedioic acid | C26 H50 O4 | -1.24 | -1.24 | 1 |
| 101 | 24.497 | 337.3344 | Cyclohexanecarbonylpentadecylamine | C22 H43 N O | 0.33 | 0.33 | 3 |

**Table 2S.** Compounds tentatively identified in RP-UHPLC-MS (negative mode) analysis of PAME.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound No** | **RT** | **Molecular****Mass** | **Identification** | **Molecular****Formula** | **MFG****Diff ppm** | **DB Diff****ppm** | **Hits** |
| 1 | 1.176 | 118.0273 |  - | C5 H2 N4 | 5.29 |  |  |
| 2 | 10.756 | 169.13 | Didox | C7 H7 N O4 | 3.59 | 3.59 | 9 |
| 3 | 12.122 | 230.023 | 5-(1-Propynyl)-5'-vinyl-2,2'-bithiophene |  |  | -2.86 | 1 |
| 4 | 12.563 | 252.0999 | Trinexapac-ethyl | C13 H16 O5 | -0.46 | -0.45 | 5 |
| 5 | 12.726 | 283.9931 | - | C9 H4 N2 O9 | -5.1 |  | 4 |
| 6 | 13.134 | 315.9987 | - | C10 H8 N2 O8 S | 4.5 |  |  |
| 7 | 14.353 | 195.0894 | O-Benzyl-L-Serine | C10 H13 N O3 | 0.55 | 0.55 | 8 |
| 8 | 15.332 | 298.1607 |  -  | C16 H26 O3 S | -1.39 |  |  |
| 9 | 15.62 | 298.1612 |  - | C16 H26 O3 S | -3.12 |  |  |
| 10 | 17.855 | 294.1882 | Tetradecyl sulfate | C14 H30 O4 S | -5.86 | -5.87 | 2 |
| 11 | 19.769 | 644.2435 |  | C34 H32 N10 O2 S | -0.65 |  |  |
| 12 | 19.773 | 523.2925 | PS(18:1(9Z)/0:0) | C24H46NO9P |  | -2.76 | 2 |
| 13 | 19.781 | 474.2928 | - | C20 H34 N12 O2 | -0.11 |  |  |
| 14 | 20.145 | 322.2219 | - | C14 H34 N4 S2 | 1.92 |  |  |

**Table 3S.** Effect of methanol extract (PAME) of *P. afra* on weight (g) of organs of albino rat.

|  |  |  |
| --- | --- | --- |
| **Organ of rat** | **Control (mg/kg)** | **MPA 5000mg/kg** |
| Kidney | 0.949±0.11 | 0.867±0.1 |
| Heart | 0.821±0.12 | 0.802±0.2 |
| Lungs | 1.75±1.1 | 1.52±1.4 |
| Liver | 7.07±2.1 | 6.63±1.2 |
| Spleen | 0.213±o.1 | 0.23±0.2 |

All values are expressed as mean ± SD., (n = 3).

**Table 4S.** Behavioral patterns in the control and treated group with crude PAME extract of *P. afra*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters** | **4 hours** | **48 hours** | **7 days** | **14 days** |
| Alertness | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Skin irritation | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Respiration | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Seizures | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Salivation | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Coma | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Mortality | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Eye lacrimation | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Diarrhea  | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Touch response  | + | + | + | + | + | + | + | + |
| Writhing reflex  | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |
| Urination  | + | + | + | + | + | + | + | + |

Where P = present, N.P = not present and N = normal.

**Table 5S. Results of PAME P. afra (5000mg/kg) for 14 days on different histopathological parameters on different organs of rats (n=6).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr. No** | **Parameters** | **Control group** | **Treatment Group**  |
| **Liver** |
| 1 | Necrosis  | - | - |
| 2 | Inflammation  | - | - |
| 3 | Fatty change  | - | - |
| 4 | Spotty necrosis | - | - |
| 5 | Ballooning | - | - |
| **Lungs** |
| 6 | Alveolar collapse | - | - |
| 7 | Macrophages  | - | - |
| 8 | Focal exudate  | - | - |
| 9 | Diffuse exudates | - | - |
| **Kidney** |
| 11 | Necrosis | - | - |
| 12 | Rupture | - | - |
| 13 | Glomerulus sclerosis | - | - |
| 14 | Interstitial inflammation | - | - |