**Supplementary Material**

**Chemical profiling of *Verbena officinalis* and assessment of its anti- cryptosporidial activity in experimentally infected immunocompromised mice**

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**Appendix**

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| **Item** | **Subject** |
| 1S | Chemical characterization of *V. officinalis* extract using LC-ESI-MS/MS |

**Chemical characterization of *V. officinalis* extract using LC-ESI-MS/MS**

A molecular ion peak was detected at Rt (0.77) with a deprotonated ion [M-H]− at *m/z* 133 and a daughter ion at *m/z* 115 [M-H-18]− due to the neutral loss of H2O moiety; it was identified as malic acid as previously described (Sobeh et al., 2018). A molecular ion peak was detected at Rt (0.98 min); it exhibited a precursor ion [M-H]- at *m/z* 179, corresponding with the molecular formula C9H7O4. Also, it produces a daughter MS fragment at *m/z* 135 due to the loss of CO2 moiety (-*m/z* 44) [M-H-CO2]-, another MS ion was detected at *m/z* 107. As previously described, this compound could be identified as caffeic acid (Ghareeb et al., 2018a; Bakchiche et al., 2019). A molecular ion peak was detected at Rt (7.25) with a deprotonated ion [M-H]− at *m/z* 367 and a daughter ion at *m/z* 191 [M-H-176]− corresponding to quinic acid deprotonated molecule and due to the neutral loss of feruloyl moiety (-176 Da). Another set of characteristic molecular ions was observed at *m/z* 179, 161, 135, and 117. In this regard, the compound was identified as feruloyl quinic acid (Abu-Reidah et al., 2013). A molecular ion peak was detected at Rt (29.71) with a deprotonated ion [M-H]− at *m/z* 415, and characteristic molecular ions were detected at *m/z* 385, 251, 165, and 147. This compound was identified from this mass fragmentation pattern as a dihydro-*p*-coumaric acid derivative (Ghareeb et al., 2018b).

A peak was detected at Rt (6.06), exhibiting a deprotonated ion [M-H]− at *m/z* 611. Also, a set of characteristic fragments were detected at *m/z* 491 [M-H-120]−, 431 [M-H-2×90]−, 401 [M-H-90-120]−, and 371 ([M-H-2×120]−, indicating to the presence of a di-*C-*hexoside moiety. This fragmentation pattern is typically assigned to eriodictyol-di-*C*-hexoside (De Beer et al., 2012). A peak was detected at Rt (6.15), exhibiting a deprotonated ion [M-H]− at *m/z* 449 and characteristic ions at *m/z* 287 corresponding to eriodictyol aglycone and due to neutral loss of glucose moiety [M-H-162]−. Other ions were detected at *m/z* 151 and 107; this fragmentation pattern is typically assigned to eriodictyol glycoside. Therefore, the compound could be identified as eriodictyol 7-*O*-glucoside (Friščić et al., 2016). A molecular ion peak was detected at Rt (6.33), exhibiting a deprotonated ion [M-H]− at *m/z* 433. It exhibited characteristic ion at *m/z* 301 corresponding to quercetin aglycone and due to neutral loss of apiose/pentose moiety [M-H-132]−. In this regard, the compound was identified as quercetin 7-*O*-pentoside/apioside(Pascale et al., 2020). A molecular ion peak was detected at Rt (7.42), exhibiting a deprotonated ion [M-H]− at *m/z* 623 and molecular ions at *m/z* 477 [M-H-Rha]− due to neutral loss of rhamnosyl moiety (-*m/z* 146 Da). Besides, a diagnostic molecular ion peak was detected at *m/z* 315 [M-H-Rha-Glu]− corresponding to isorhamnetin aglycone anddueto furtherneutral loss of glucose moiety (-*m/z* 162 Da). Another fragment was observed at *m/z* 300 [M-H-Rha-Glu-CH3]− due to further neutral loss of methyl group (-*m/z* 15 Da). In this regard, the compound was identified as isorhamnetin-3-*O-*rutinoside(Al-Yousef et al., 2020).

A molecular ion peak was detected at Rt (8.04), exhibiting a deprotonated ion [M-H]− at *m/z* 445 and molecular ions at *m/z* 269 corresponding to apigenin aglycone and due to neutral loss of glucuronide moiety (-176Da). This compound was identified from this mass fragmentation pattern as Apigenin 7-*O*-glucuronide (Friščić et al., 2016). A molecular ion peak was detected at Rt (8.71), exhibiting a deprotonated ion [M-H]− at *m/z* 621 and a characteristic molecular ion at *m/z* 269 corresponding to apigenin aglycone and due to neutral loss of two glucuronide moieties (-2 X 176Da). From this mass fragmentation pattern, this compound was identified as Apigenin 7-*O*-diglucuronide(Rehecho et al., 2011). A molecular ion peak was detected at Rt (8.85), exhibiting a deprotonated ion [M-H]− at *m/z* 301. Additionally, characteristic molecular ions were detected at *m/z* 300, 271, 269, 255, 229, 179, 169, and 151. From this mass fragmentation pattern, this compound was identified as Quercetin (Ghareeb et al., 2018a). A molecular ion peak was detected at Rt (20.43), exhibiting a deprotonated ion [M-H]− at *m/z* 447, and a characteristic molecular ion as detected at *m/z* 285 [M-H-162]− corresponding to kaempferol aglycone and dueneutral loss of glucoside moiety (-162Da). Other diagnostic fragments for kaempferol aglycone (Ghareeb et al. 2018c) were also detected at *m/z* 284, 255, 227, and 151. From this mass fragmentation pattern, this compound was identified as Kaempferol-3-*O*-glucoside (Ghareeb et al., 2018a).

A molecular ion peak was detected at Rt (29.32), exhibiting a deprotonated ion [M-H]− at *m/z* 255, and characteristic molecular ions were detected at *m/z* 213, 183, and 172. From this mass fragmentation pattern, this compound was identified as Pinocembrin(Simirgiotis et al., 2015; Ghareeb et al., 2018c). A molecular ion peak was detected at Rt (29.75) with a deprotonated ion [M-H]− at *m/z* 461, and characteristic molecular ions were detected at *m/z* 341 [M\_H-120], 311 [M-H-150], and 299 [M-H-162]-. This mass fragmentation pattern identified this compound as Chrysoeriol 8-*C*-glucoside (Scoparin)(Hassan et al., 2019).

A molecular ion peak was detected at Rt (7.69), exhibiting a deprotonated ion [M-H]− at *m/z* 623 and molecular ions at *m/z* 461 [M-H-caffeic acid moiety]− due to neutral loss of caffeic acid moiety (-*m/z* 162 Da), and a diagnostic molecular ion at *m/z* 315 [M-H-anhydrorhamnosyl]− dueto furtherneutral loss of rhamnosyl moiety (-*m/z* 146 Da) and corresponding to hydroxytyrosol glucoside (*m/z* 315). Therefore, the compound was identified as verbascoside (acteoside)(Friščić et al., 2016). A molecular ion peak was detected at Rt (8.99) with a molecular ion at *m/z* 177 [M-H]−, it showed characteristic fragment *m/z* 133[M-H-44]- due to neutral loss of CO2 moiety (-44Da). In this regard, the compound was identified as Esculetin(Li et al., 2012).