Phytochemical study and anti-inflammatory activity of the roots of \textit{Mangifera indica} L. in lipopolysaccharide (LPS)-stimulated peritoneal macrophages

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\textbf{ABSTRACT}

This study deals with the identification of secondary metabolites of methanol extract of the roots of \textit{Mangifera indica} L and the assessment of plant \textit{in vitro} anti-inflammatory activity. High-performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC-ESI-MS\textsuperscript{n}) using the negative ion mode was performed to establish the chromatographic fingerprint and identify various chemical components of the plant extract. The anti-inflammatory effect of the MeOH extract (3, 30 and 300 µg/mL) was assessed through cell viability and nitric oxide (NO) production on non-stimulated and LPS-stimulated peritoneal macrophages. Phytochemical analysis indicated the presence of a number of phenolic compounds where galloyl derivatives, mangiferin and its derivatives were the major constituents. The methanol extract exhibited significant concentration-dependent inhibitory effect on NO production, both on stimulated and non-stimulated macrophages. The concentration 300 µg/mL showed significant cell toxicity. The methanol extract of \textit{Mangifera indica} is rich in phenolic compounds and possesses potent \textit{in vitro} anti-inflammatory activity, but its higher concentrations are cytotoxic.

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\textbf{1. Introduction}

Inflammation is a biological reaction that occurs in response to tissue damage with the aim to remove harmful stimuli, including damaged cells, pathogens, or endotoxins like lipopolysaccharides (LPS) (Cheng \textit{et al.}, 2014). Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa \textit{et al.}, 2002). Indeed, inflammatory mediators such as reactive oxygen species (ROS), nitric oxide (NO), and interleukins (IL-1, IL-6) are overproduced by macrophages under inflammatory conditions. High concentrations of these mediators have been implicated in the pathological processes of chronic diseases including rheumatoid arthritis, cardiovascular diseases, chronic hepatitis, pulmonary fibrosis and inflammatory brain disease (Wang and Mazza, 2002). Therefore, the inhibition of overproduction of inflammatory mediators could be of great help in the treatment of many diseases.

Till date, 74% of pharmacologically active plant-derived components have been discovered after following up on ethnomedical use of plants (Farnsworth and Soejarto, 1991). Previous reports have shown that medicinal plants possess a large number of secondary metabolites, belonging to different classes of natural products possessing interesting biological activities (Frezza \textit{et al.}, 2017; Mohammadhosseini \textit{et al.}, 2019). General correlations between the ethnopharmacological uses and medicinal properties identified through systematic research have been
observed (Mohammadhosseini et al., 2019). Moreover, some of the medicinal properties could also be linked to the phytochemicals present in the plant extract (Magne et al., 2017; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017).

The different chemical constituents of Mangifera indica L. are mainly polyphenolics and triterpenoids. Mangiferin, a xanthone glycoside, is a major bioactive constituent (Scartezzini and Speroni, 2000). It has been reported that different parts (stem bark, leaves and fruit) of this species possessed antioxidiant (Pardo-Andreu et al., 2006; Rocha Ribeiro et al., 2007), antidiabetic (Sharma et al., 1997; Aderibigbe et al., 1999), hepatoprotective (Prasad et al., 2007), analgesic, and anti-inflammatory activities (Garrido et al., 2001).

Although a lot of pharmacological investigations have been carried out based on the present secondary metabolites, the effect of MeOH extract of the roots of M. indica, in association with LPS-induced inflammatory and NO production in peritoneal macrophage, has not been explored. Therefore, in this study, we attempted to determine the underlying mechanisms of its anti-inflammatory activities and the main compounds present in the extract.

2. Materials and Methods

2.1. Plant material

The roots of Mangifera indica L. were collected in February 2017 at Siteu-Dschang, in West region, Cameroon (05º35.911’N 010º41.346’E at 1138 meter altitude), identified and authenticated by Nole Tsabang by comparison with a voucher specimen Nº 2015HN/CAM deposited at the National Herbarium (Yaounde, Cameroon).

2.2. Phytochemical analysis

2.2.1. Chemical reagents

All reagents and standards were of analytical reagent grade. For phytochemical analysis, all standards were purchased from Sigma-Aldrich (Madrid, Spain). Individual stock solutions were prepared in ethanol (HPLC grade; Sigma). LC-MS grade acetonitrile (Sigma) and ultrapure water (Milli-Q Waters purification system; Millipore; Milford, MA, USA) were also used. N(ω)-nitro-L-arginine methyl ester (L-NAME) L-NAME, lipopolysaccharides (LPS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide, sulfanilamide, H3PO4, naphthylethylenediamine dihydrochloride were purchased from Sigma-Aldrich (Germany).

2.2.2. Preparation of extract

The dried roots of M. indica were ground into powder. The obtained powder (4 Kg) was macerated for 48 h in MeOH at room temperature and the resulting mixture was filtrated. The filtrate was concentrated under vacuum on a rotator evaporator at 40 ºC, obtaining a viscous residue that was freeze-dried, and yielded 227 g of dried extract.

2.2.3. Chromatographic conditions

For high-performance liquid chromatography analysis, 5 mg of dried extract was re-dissolved in 1 mL of MeOH. After filtration through 0.45 μm PTFE membrane filters, 10 μL of sample was injected.

The HPLC system was an Agilent Series 1100 with a G1315B diode array detector (Agilent Technologies, Santa Clara, CA, USA). The separation of the compounds was performed with a reversed phase Luna Omega Polar C18 analytical column of 150 x 3.0 mm and 5 μm particle size (Phenomenex, Torrance, CA, USA). A polar C18 security guard cartridge (Phenomenex) of 4 x 3.0 mm was also used. The high-performance liquid chromatography system was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray interface.

The high-performance liquid chromatography system was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray interface operating in negative ion mode. The scan range was set at m/z 100-1200 with a speed of 13,000 Da/s. The ESI conditions were as follows: drying gas (N2) flow rate and temperature, 10 mL/min and 365 ºC; nebulizer gas (N2) pressure, 50 psi; capillary voltage, 4500 V; capillary exit voltage, -117.3 V. The auto MS² mode was used for the acquisition, with isolation width of 4.0 m/z, and fragmentation amplitude of 0.6 V (MS² up to MS³). Esquire control software was used for the data acquisition and data analysis for processing (Llorent-Martinez et al., 2018).

The analysis of the phenolic composition of the roots of M. indica was carried out by HPLC-ESI-MS² using the negative ionization mode. The initial step for the characterization of the different phenolic compounds in the analyzed samples consisted of the determination of the molecular weight of each compound. In the negative ionization mode (ESI) MS² spectrum, the most intense peak corresponded to the deprotonated molecular ion [M-H]⁻.

2.3. Biological activities

Prior authorization for the use of laboratory animal was obtained from the National Ethical Committee (Ref. Nº CEI- 2018/1048).

2.3.1. Peritoneal macrophage cell culture
Rats were sacrificed by cervical dislocation and cells were collected through washing the peritoneum with cold phosphate buffered saline (PBS)-EDTA solution. The collected fluid was centrifuged at 1500 rpm and the supernatant discarded. The pellet was washed twice with PBS by centrifugation. Then, the washed cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin and incubated at 37 °C in an humidified environment with 5% of CO₂ for 3 h to eliminate death cells and non-macrophage cells. The remaining macrophages were counted and sealed at density of 10⁵ per well in 96 wells microplate for 2 h.

2.3.2. Effect of extract on NO production and cell viability

The macrophages were exposed to plant extracts at concentrations of 0, 3, 30, or 300 µg/mL. In another set of experiments, designed to mimic the inflammatory condition, macrophages were stimulated by adding LPS (1 µg/mL) in the culture medium, 15 min after plant extract or L-NAME. After 8 hours of incubation in both protocols, cell cultured media were collected for nitric oxide determination. The attached cells were used for the estimation of cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

2.3.3. Determination of cell viability

The macrophages previously incubated with plant extracts or DMSO (vehicle) were washed several times with PBS to remove any residual extract color that may interfere with the colorimetric assay. The extract-free cells were finally incubated in 120 µL media containing MTT (0.03%) for 3 hours at 37 °C in 5% CO₂ humidified incubator. Wells were then washed several times and 150 µL of DMSO was added. The absorbance of the produced intracellular formazan, which is proportional to the number of present viable cells, was determined at 620 nm. Wells filled with media alone was used as a blank.

2.3.4. Nitrite determination

The stable product of NO oxidation, nitrite, was measured using the Griess reaction. Samples (200 µL) were mixed with an equal volume of the Griess reagent (2% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride) and incubated at room temperature for 10 min. The absorbance of each sample was measured spectrophotometrically at 540 nm and NO concentrations were calculated using standard curve of sodium nitrite.

2.4. Statistical analysis

Data are presented as means ± standard error of the mean (S.E.M.). For statistical evaluation, comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls method.

3. Results and Discussion

3.1. Phytochemical profiles

We performed the identification of the compounds by HPLC-ESI-MS⁻ using the negative ion mode, using analytical standards and bibliographic data. The characterization of phytochemicals is shown in Table 1, whereas the base chromatogram of the MeOH crude extract is shown in Fig. 1.

3.2. Phenolic acids

Compound 6 was identified as gallic acid by comparison with an analytical standard (169→125 fragmentation). Compound 4 suffered the neutral loss of 162 Da, yielding gallic acid; this fragmentation was consistent with galloyl-glucose. Similarly, compound 12 was characterized as methyl gallate due to the loss of a methyl group. Compound 8 was identified as protocatechuic acid by comparison with an analytical standard. Compounds 17, 22, and 28, which have been previously identified in M. indica, were tentatively characterized as trigalloyl-, tetragalloyl-, etc.
### Table 1

Characterization of the compounds found in the methanol extract of the roots of *Mangifera indica*.

<table>
<thead>
<tr>
<th>No.</th>
<th>tᵣ (min)</th>
<th>[M-H] m/z</th>
<th>m/z (% base peak)</th>
<th>Assigned identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
<td>377</td>
<td></td>
<td><strong>Disaccharide (chloride adduct)</strong></td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>575</td>
<td></td>
<td>Maclinor galloyl-gluccoside</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>421</td>
<td>331 (68), 301 (100), 271 (2)</td>
<td>Mangiferin isomer</td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
<td>331</td>
<td></td>
<td>Galloyl-glucose</td>
</tr>
<tr>
<td>5</td>
<td>2.7</td>
<td>343</td>
<td>193 (100), 169 (84), 125 (10)</td>
<td>Galloyquinic acid</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>169</td>
<td></td>
<td>Gallic acid*</td>
</tr>
<tr>
<td>7</td>
<td>4.2</td>
<td>423</td>
<td>303 (100), 193 (7)</td>
<td>Maclinor-C-glucoside</td>
</tr>
<tr>
<td>8</td>
<td>5.3</td>
<td>153</td>
<td></td>
<td>Protocatechuic acid*</td>
</tr>
<tr>
<td>9</td>
<td>6.2</td>
<td>583</td>
<td><strong>MS² [583]: 565 (28), 493 (100), 463 (75), 423 (24), 333 (15), 301 (42), 271 (5)</strong></td>
<td>Mangiferin-C-gluccoside</td>
</tr>
<tr>
<td>10</td>
<td>6.9</td>
<td>575</td>
<td><strong>MS² [575]: 423 (24), 333 (6), 313 (35), 301 (100), 285 (64), 193 (35)</strong></td>
<td>Maclinor galloyl-gluccoside</td>
</tr>
<tr>
<td>11</td>
<td>7.2</td>
<td>407</td>
<td><strong>MS² [407]: 317 (10), 287 (100), 245 (2)</strong></td>
<td>Iriphlofenone-3-C-β-D-gluccoside</td>
</tr>
<tr>
<td>12</td>
<td>8.2</td>
<td>183</td>
<td><strong>MS² [183]: 183 (100), 168 (22), 124 (25)</strong></td>
<td>Methyl gallate</td>
</tr>
<tr>
<td>13</td>
<td>8.2</td>
<td>289</td>
<td><strong>MS² [289]: 245 (100), 205 (29), 203 (22), 179 (12)</strong></td>
<td>Catechin*</td>
</tr>
<tr>
<td>14</td>
<td>10.7</td>
<td>421</td>
<td><strong>MS² [421]: 331 (57), 301 (100), 271 (7)</strong></td>
<td>Mangiferin isomer</td>
</tr>
<tr>
<td>15</td>
<td>10.7</td>
<td>559</td>
<td><strong>MS² [559]: 407 (100), 389 (10), 317 (10), 285 (95), 269 (87), 245 (10), 169 (7)</strong></td>
<td>Iriphlofenone-3-C-(2-O-p-hydroxybenzoyl)-β-D-gluccoside</td>
</tr>
<tr>
<td>16</td>
<td>11.3</td>
<td>727</td>
<td><strong>MS² [727]: 575 (100), 557 (9), 465 (25), 423 (5), 333 (7), 315 (8), 295 (16)</strong></td>
<td>Iriphlofenone 3-C-(2,6-di-O-gallloyl)-β-D-gluccoside</td>
</tr>
<tr>
<td>17</td>
<td>11.5</td>
<td>635</td>
<td><strong>MS² [635]: 465 (100)</strong></td>
<td>Trigallaryl glucoside</td>
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<tr>
<td>18</td>
<td>12.0</td>
<td>589</td>
<td><strong>MS² [589]: 437 (18), 419 (100), 317 (26), 299 (88), 169 (15)</strong></td>
<td>Galloylglucoside</td>
</tr>
<tr>
<td>19</td>
<td>12.5</td>
<td>435</td>
<td><strong>MS² [435]: 315 (100), 287 (51), 272 (51)</strong></td>
<td>Unknown</td>
</tr>
<tr>
<td>20</td>
<td>14.8</td>
<td>573</td>
<td><strong>MS² [573]: 421 (100), 313 (15), 301 (26), 285 (10)</strong></td>
<td>Methoxy-mangiferin</td>
</tr>
<tr>
<td>21</td>
<td>15.7</td>
<td>573</td>
<td><strong>MS² [573]: 421 (100), 301 (29), 283 (74), 259 (28)</strong></td>
<td>Mangiferin gallate</td>
</tr>
<tr>
<td>22</td>
<td>16.9</td>
<td>787</td>
<td><strong>MS² [787]: 635 (100), 617 (28), 465 (8)</strong></td>
<td>Mangiferin gallate</td>
</tr>
<tr>
<td>23</td>
<td>19.0</td>
<td>725</td>
<td><strong>MS² [725]: 573 (100), 421 (17), 416 (53)</strong></td>
<td>Tetragallaryl-glucoside</td>
</tr>
<tr>
<td>24</td>
<td>19.0</td>
<td>693</td>
<td><strong>MS² [693]: 573 (26), 541 (36), 421 (100), 313 (44), 301 (73)</strong></td>
<td>Mangiferin gallate derivative</td>
</tr>
<tr>
<td>25</td>
<td>19.3</td>
<td>303</td>
<td><strong>MS² [303]: 285 (100), 177 (17)</strong></td>
<td>Taxifolin</td>
</tr>
<tr>
<td>26</td>
<td>21.0</td>
<td>541</td>
<td><strong>MS² [541]: 421 (19), 403 (80), 283 (100)</strong></td>
<td>Unknown</td>
</tr>
<tr>
<td>27</td>
<td>21.5</td>
<td>573</td>
<td><strong>MS² [573]: 555 (30), 421 (14), 403 (10), 301 (91)</strong></td>
<td>Mangiferin gallate</td>
</tr>
<tr>
<td>28</td>
<td>21.6</td>
<td>939</td>
<td><strong>MS² [939]: 787 (11), 769 (100), 617 (8)</strong></td>
<td>Pentagallarlyl-glucoside</td>
</tr>
<tr>
<td>29</td>
<td>22.8</td>
<td>723</td>
<td><strong>MS² [723]: 677 (100)</strong></td>
<td>Unknown</td>
</tr>
<tr>
<td>30</td>
<td>24.4</td>
<td>285</td>
<td><strong>MS² [285]: 285 (100), 241 (15), 175 (10)</strong></td>
<td>Unknown</td>
</tr>
<tr>
<td>31</td>
<td>25.0</td>
<td>543</td>
<td><strong>MS² [543]: 403 (16), 271 (100)</strong></td>
<td>Dihydrokaempferol</td>
</tr>
<tr>
<td>32</td>
<td>25.3</td>
<td>287</td>
<td><strong>MS² [287]: 259 (100), 243 (11), 153 (3)</strong></td>
<td>Unkonwn</td>
</tr>
<tr>
<td>33</td>
<td>26.4</td>
<td>613</td>
<td><strong>MS² [613]: 305 (221), 261 (65), 179 (100), 125 (54)</strong></td>
<td>Unknown</td>
</tr>
<tr>
<td>34</td>
<td>26.4</td>
<td>285</td>
<td><strong>MS² [285]: 257 (11), 163 (100), 135 (61)</strong></td>
<td>Unknown</td>
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<tr>
<td>35</td>
<td>27.2</td>
<td>433</td>
<td><strong>MS² [433]: 271 (100)</strong></td>
<td>Trihydroxyflavanone-O-hexoside</td>
</tr>
<tr>
<td>36</td>
<td>31.9</td>
<td>287</td>
<td><strong>MS² [287]: 151 (100), 135 (7)</strong></td>
<td>Enidictyol</td>
</tr>
<tr>
<td>37</td>
<td>34.3</td>
<td>301</td>
<td><strong>MS² [301]: 179 (100), 151 (68)</strong></td>
<td>Quecostein*</td>
</tr>
<tr>
<td>38</td>
<td>35.1</td>
<td>627</td>
<td><strong>MS² [627]: 253 (88), 153 (11), 135 (100)</strong></td>
<td>Trihydroxyflavanone</td>
</tr>
<tr>
<td>39</td>
<td>38.4</td>
<td>271</td>
<td><strong>MS² [271]: 253 (88), 153 (11), 135 (100)</strong></td>
<td>Trihydroxyflavanone</td>
</tr>
<tr>
<td>40</td>
<td>40.0</td>
<td>759</td>
<td><strong>MS² [759]: 613 (100), 595 (20), 453 (12), 305 (29)</strong></td>
<td>(Epigallicatechin glycoside)</td>
</tr>
</tbody>
</table>

*Identified by comparison with analytical standards.*
and pentagalloyl-glucosides, respectively, based on bibliographic data (Gómez-Caravaca et al., 2016; Dorta et al., 2014). Finally, we identified compound 5 as galloylquinic acid due to the fragment ions at m/z 191 (quinic acid) and 169 (gallic acid).

3.3. Flavonoids

Compound 13 was unambiguously identified as catechin by comparison with an analytical standard. Compound 25, with [M-H] at m/z 303 and fragment ions at m/z 285 and 177, were characterized as taxifolin (Mämmelä, 2001). Although 6-(p-hydroxybenzyl)taxifolin-7-O-β-D-glucoside (tricuspid) has been reported in M. indica (Kanwal et al., 2010), this is the first report of the aglycone taxifolin in M. indica to our best knowledge.

Other flavonoids – as aglycones – were also identified. Compound 32, commonly known as aromadendrin, was dihydrokaempferol (287→259 fragmentation) (Marles et al., 2003). Compound 36 corresponded to eriodictyol due to the 287→151 fragmentation (Justesen, 2000), whereas compound 37 was identified as quercetin by comparison with an analytical standard. To our best knowledge, dihydrokaempferol and eriodictyol have not been previously reported in M. indica extracts.

Compound 39 exhibited the deprotonated molecular ion at m/z 271 and presented fragment ions at m/z 253, 153, and 135. This behavior has been reported for trihydroxyflavanones (Ye et al., 2012). With an additional hexose moiety (162 Da), compound 35 was characterized as trihydroxyflavanone-O-hexoside.

Finally, compound 40 displayed [M-H] at m/z 759 and suffered neutral losses of 146, 146, and 162 Da, yielding gallicatechin at m/z 305 (Sun et al., 2007). This fragmentation corresponds to gallicatechin-dicoumarol (hexoside), although the exact isomer could be elucidated.

3.4. Benzophenone derivatives

Several benzophenones, major intermediates in the biosynthetic pathway of xanthones, were also characterized in the MeOH extract of M. indica.

Three maclurin derivatives were tentatively characterized. Compounds 2 and 10 exhibited [M-H] at m/z 575, and similar fragmentations. These fragmentation patterns have been previously reported for maclurin galloyl-glucoiside (Dorta et al., 2014; Gómez-Caravaca et al., 2016) in M. indica extracts. The fragmentation pattern of compound 7 was in agreement with maclurin-C-glucoside (Gómez-Caravaca et al., 2016).

Compounds 11, 15, and 16 were tentatively characterized as iriflophenone derivatives by comparison their deprotonated molecular ions and fragmentation patterns with bibliographic information (Barreto et al., 2008).

3.5. Xanthones and derivatives

Compounds 3 and 14 exhibited [M-H] at m/z 421, and fragment ions at m/z 331, 301, and 271. This fragmentation pattern is consistent with the xanthone-C-glycoside mangiferin (Barreto et al., 2008). Considering the order of elution (Barreto et al., 2008), compound 3 could correspond to isomangiferin and 14 to mangiferin. However, in the absence of analytical standard, we characterized both compounds as mangiferin isomers. Compound 9 presented [M-H] at m/z 583, 162 Da higher than mangiferin. It displayed neutral losses of 18, 90, and 120 Da, consistent with C-glycosides. Hence, it was characterized as mangiferin-C-hexoside.

Compound 19, with an extra methyl group than mangiferin, was tentatively characterized as methoxy-mangiferin (Barreto et al., 2008; Gómez-Caravaca et al., 2016). Compounds 20, 21, and 27 suffered the neutral loss of a galloyl moiety (152 Da) to yield mangiferin at m/z 421. Hence, we characterized them as mangiferin gallate isomers. With an additional galloyl moiety, compound 23 was characterized as mangiferin digallate. Compound 24 was a derivative of mangiferin gallate.

3.6. Other compounds

Compound 1 presented fragment ions typical from hexoses at m/z 179, 161, 143, 131, 119, and 113, corresponding to a disaccharide containing two hexoses, probably glucosides (Verardo et al., 2009).

From the HPLC-ESI/MS' base peak chromatogram (BPC), the major compounds of the MeOH extract of M. indica seem to be mangiferin, maclurin galloyl-glucoside, mangiferin gallate and (epi)gallicatechin glycoside. The structures of mangiferin and maclurin are shown in Fig. 2.

![Fig. 2. The structures of major compounds of MeOH extract of the roots of Mangifera Indica.](image)

Fig. 2. The structures of major compounds of MeOH extract of the roots of Mangifera Indica.
3.7. Effects of the extract on production of NO in peritoneal macrophage cells

Although NO is responsible for the host defense mechanism (Boscá et al., 2005), a high level of NO concentration can cause toxicity and damage to host cells. Excessive NO production is involved in various inflammatory diseases (Shaw et al., 2005; Förstermann, 2010) due to overexpression of both iNOS and COX2, enzymes which are commonly associated with inflammation. It has been reported that NO is a major inflammatory mediator. In this study, the influence of the extract on the production of NO by the peritoneal macrophage cells has been investigated. The increase (p<0.05) production of NO was detected following the treatment of the macrophage cells with Mangifera indica (MI) extract at a concentration of 3 µg/mL (Fig. 3A). The treatment of peritoneal macrophage cells with the highest concentrations of MI extract led to an inhibition of the production of NO with a significant value at 300 µg/mL (Fig. 3A). These results imply that in normal conditions, low concentration of the MeOH extract of Mangifera indica may potentiate NO production. Therefore, indicating that Mangifera indica extract can enhance normal cell mediation and help in the treatment of diseases such as arterial hypertension. Macrophages play an important role in the activation and release of the pro-inflammatory mediators and cytokines, including NO, IL-1β, and IL-6, when treated by LPS. LPS is the thicker layer of LPS at the outer membrane of the cell wall of Gram-negative bacteria (Cuschierei and Maier, 2007; Lucas and Maes, 2013). The process may be followed by the production of pro-inflammatory mediators, cytotoxicity, and apoptosis (Shu et al., 2016). Indeed, the level of NO increased significantly in LPS-stimulated cells when compared with the normal control group. Mangifera indica extract were tested at all the concentrations (3-300 µg/mL). Moreover, L-NAME, a strong iNOS inhibitor, caused a significantly and concentration-dependently reduce in the NO overproduction of LPS stimulated macrophages (Fig. 3B). This suggests that Mangifera indica extract may interfere with the signaling pathway of LPS-induced NO production.

3.8. Effect of extract on Cell Viability

The cytotoxicity of the extract of MI (3-300 µg/mL) on peritoneal macrophage cells was measured by MTT assay in the presence or in absence of LPS. Compared with the vehicle controls, the results of the MTT assay demonstrated that the extract of MI at the concentration of 3 and 30 µg/mL had no cell toxicity. The maximum concentration (300 µg/mL) tested showed a significant (p<0.001) decrease in cell viability as compared with the control (Fig. 4A), indicating cell toxicity. In the presence of LPS, cell toxicity was observed only at the highest concentration (300 µg/mL; p<0.001) of MI extract (Fig. 4B).

The lower concentrations did not affect the cell viability; instead, they showed an increase in the viability of the peritoneal macrophages. However, at the highest concentration (300 µg/mL), the viability was significantly reduced. Similar results were obtained by Baskaran et al. (2017) on the cell viability test of the RAW 264.7 macrophages. The cytotoxicity of the concentration 300 µg/mL of the extract of MI could be due to the oversaturation of compounds in that particular concentration, which led the cells to have a reduced viability (Baskaran et al., 2017).

From the phytochemical analysis, the MeOH extract of MI contains a number of phenolic compounds, which have been previously reported (Masibo and He 2008). The major polyphenols identified in the MeOH extract of MI were mangiferin, mangiferin derivatives, galloyl derivatives, and flavonoids involving quercetin, dihydrokaempferol, trihydroxyflavanone, and trihydroxyflavanone-O-hexoside. Different biological activities have been reported for these phenolic compounds. Mangiferin demonstrated anti-inflammatory abilities by modulating several key inflammatory pathways (Vyas et al., 2012). It has also been shown to be an effective inhibitor of NF-κB signaling pathway (Vyas et al., 2012). Mangiferin plays an important role in the balance between the overwhelming anti-inflammatory cytokines and
pro-inflammatory mediators, by the inhibition of inflammatory cellular activations (Sánchez et al., 2000; Garrido et al., 2006). It has been reported to significantly reduce the production of pro-inflammatory mediators (COX-2, iNOS and TNF-α) in LPS stimulated RAW 264.7 cells (Bulugonda et al., 2017). Other phenolic compounds like galloyl derivatives found in this extract have been reported to possess strong inhibitory activities against inflammatory cellular activations (Sánchez et al., 2000; Garrido et al., 2001; Denegri et al., 2004). It has been reported to significantly reduce NO production in LPS-stimulated RAW264.7 cells (Keuls).

### Cell viability on non-stimulated (A) and lipopolysaccharide (MI) and N(ω)-nitro-L-arginine methyl ester (L-NAME) on (B) peritoneal macrophages. The values are expressed as means ± SEM of five independent experiments. ***p<0.001 significantly different as compared with control group; ###p<0.001 significantly different as compared with the LPS group (one way ANOVA followed by Student-Newman-Keuls).

#### Fig. 4. Effect of MeOH extract of the roots of Mangifera indica (MI) and N(ω)-nitro-L-arginine methyl ester (L-NAME) on cell viability on non-stimulated (A) and lipopolysaccharide (LPS)-stimulated (B) peritoneal macrophages. The values are expressed as means ± SEM of five independent experiments.

#### References


4. Concluding remarks

Taken together, these results suggest that the roots of Mangifera indica contain potent anti-inflammatory compounds due to their overwhelming content in phenolic compounds, which reduce the production of inflammatory mediators and hold promise in the development of herbal-based anti-inflammatory therapeutics in the future.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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