Saponins are naturally occurring metabolites associated with several health benefits. The objective was to quantify and purify saponins from mate dry leaves, and to assess their anti-inflammatory and apoptotic mechanisms in human colon cancer cells in vitro. Matesaponins were extracted with methanol from dry leaves, partially purified and quantified. Leaves contained 10–15 mg/g dry weight total saponins, predominantly matesaponins 1 and 2. HPLC and LC/ESI-MS-MS identified saponins in six preparative chromatographic fractions (A, B, C, D, E, and F). Major matesaponins were identified as 1 [M–H]− = 911 and 2 [M–H]− = 1057, with trace amounts of 3 [M–H]− = 1073, 4 [M–H]− = 1219, and 5 [M–H]− = 1383. Fractions D, E, and F significantly inhibited iNOS (IC35 = 36.3, 29.5, 43.7 μM) and PGE2 (IC35 = 23.1, 22.3, 11.7 μM) and COX-2 (IC35 = 45.7, 32.4, 17.0 μM). Fraction F reduced nuclear translocation of nuclear factor-kB subunits p50 (49.8%) and p65 (49.0%) and induced apoptosis through suppression of Bcl-2 and increased Bax protein expressions and activated caspase-3 activity. Saponins in leaves of mate prevent inflammation and colon cancer in vitro.

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inflammatory agent nitric oxide (NO) (Fukumura, Kashiwagi, & Jain, 2006), a diatomic free radical, and NO-derived reactive nitrogen species, inducing oxidative and nitrosative stress are considered as pro-inflammatory mediators which lead to DNA damage (Yang, Taboada, & Liao, 2009). Prostaglandin E₂ (PGE₂) is a pro-inflammatory mediator generated at inflammatory sites by COX-2 and contributes to the development of chronic inflammatory diseases, such as cardiovascular disease and cancer (Rocca & Fitz-Gerald, 2002; Turini & DuBois, 2002). The use of anti-inflammatory drugs, which inhibit COX-2 activity, has been shown to be beneficial in preventing and treating these diseases (Bertolini, Ottani, & Sandrini, 2002; Rocca & Fitz-Gerald, 2002). Inhibition of pro-inflammatory cytokines and inhibition of NF-κB activation or function are important targets for anti-inflammatory drug development. Pro-inflammatory cytokines can be detected in colorectal cancer. There is now evidence that inflammatory cytokines, which can be produced by the tumour cells and/or tumour-associated leucocytes and platelets, may contribute directly to malignant progression. Examples are tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 (Balkwill et al., 2005; Bertolini & Ottani, 2002; Fukumura et al., 2006; Lu et al., 2006; Rocca & FitzGerald, 2002; Turini & DuBois, 2002; Waes, 2007; Yang & Taboada, 2009). Recently, it has been suggested that anti-inflammatory capacities of bioactive compounds could contribute to cancer prevention. Nevertheless, the mechanisms of anti-cancer capacity remain poorly understood.

We hypothesised that saponins purified from mate leaves will prevent colorectal carcinogenesis by suppressing inflammation and promoting apoptosis. To test this hypothesis, RAW264.7 macrophage cells were treated with different concentrations of purified saponins from mate leaves, inflammation biomarkers such as COX-2 and iNOS expression, concentration of PGE₂, NO and activation of NF-κB pathway were all measured. Moreover, we measured the anti-colon cancer potential of saponins using HT-29 colon cancer cells. Their capability to promote apoptosis was also measured. Cell cytotoxicity was performed and apoptosis was evaluated by expression of proteins associated to apoptosis, such as caspase-3 activity.

In the present study, we have identified, quantified and purified saponins from mate dry leaves, assessed their anti-inflammatory action and the corresponding underlying mechanism in vitro. We also investigated the mechanism of apoptosis inducing activity of mate saponins in human colon cancer cells.

2. Materials and methods

2.1. Chemicals

Sodium nitrite, sulphanilamide, N-1-(naphthyl) ethylenedia- mine-dihHCl and lipopolysaccharide (LPS) from Escherichia coli O55:B5) were purchased from Sigma (St. Louis, MO, USA). Macrophage RAW 264.7 cell line, colon cancer cell line HT-29, normal colon fibroblast CCD-33Co, McCoy 5A medium, Eagle’s Minimum Essential Medium and 0.25% (w/v) Trypsin- 0.53 mM EDTA and Dulbecco’s Modified Eagle Medium with L-glutamine (DMEM) were purchased from American Type Culture Collection (Manassas, VA). Faetal bovine serum was purchased from Invitrogen (Grand Island, NY, USA). Cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), NF-κB p50, NF-κB p65, Bcl-2, Bax, and actin mouse monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and antimouse IgG conjugated horseradish peroxidase secondary antibody was purchased from GE Healthcare (Buckinghamshire, UK). All other chemicals were purchased from Sigma, unless otherwise specified.

2.2. Extraction and purification of mate saponins

Saponins were extracted and fractionated from mate (Ilex paraguariensis) leaves, organically grown in Paraguay following the method by Berhow, Kong, Vermillion, and Duval (2006). Briefly, 1 kg of powdered Mate dry leaves was extracted with methanol in a Soxhlet extractor for 72 h. The extracts were reduced by rota-evaporation (40 °C), pooled and concentrated by evaporation (37 °C) under the hood. The dried material was re-suspended in methanol for preparative flash chromatography.

2.3. Preparative chromatography for purification of saponins

Six Fractions (A, B, C, D, E, F) were collected from preparative flash chromatography based on UV absorbance peaks at 210 nm. Fractions as follows: A (900 mg; eluted between 5 and 15 min), B (250 mg; eluted between 15 and 20 min), C (120 mg; eluted between 20 and 25 min), D (100 mg; eluted between 25 and 30 min), E (80 mg; eluted between 30 and 35 min), and F (75 mg; eluted between 35 and 45 min).

2.4. Analytical HPLC methodology

Mate saponin fractions D, E and F were dissolved in methanol at 2 mg/ml. An aliquot was then removed from the vial and filtered through a 0.45 μm nylon 66 filter for HPLC analysis of saponins. HPLC analysis was conducted using a Shimadzu (Columbia, MD) HPLC System (two LC 20AD pumps; SIL 20A autoinjector; DGU 20As degasser; SPD-20A UV–VIS detector; and a CBM-20A communication BUS module) running under the Shimadzu EZStart Version 7.3 software. The column was a C18 Inertsil reverse phase column (250 × 4.6 mm; RP C-18, ODS-3, 5 μm; with a Metaguard guard column; Varian, Torrance, CA). Initial chromatographic conditions were 30% acetonitrile and 0.025% trifluoroacetic acid (TFA) in water, at a flow rate of 1 ml per minute. The effluent was monitored at 210 nm. After injection (typically 25 μl), the column was developed to 50% acetonitrile and 0.025% TFA in a linear gradient over 45 min. A standard curve, based on nanomoles injected, was prepared using a purified soyasaponin I as standard. Matesaponins, for cell culture studies, were prepared by weight using 10 mg/ml DMSO as an initial stock and then diluted the initial stock with culture medium to different concentrations (1–100 μM) using the molecular weight of saponins equal to 1000, to calculate μM equivalent concentrations.

2.5. LC/ESI-MS analysis for confirmation of saponins

To confirm the identity of the matesaponins found in the mate extract fractions, aliquots were injected on a Q-TOF LC-MS. Samples were run on an Applied Biosystems/MDS Sciex QStar Elite Q-TOF mass spectrometer (MS) with a Turbo ionspray electrospray source, and a Agilent 1100 series HPLC system (G1379A degasser, G1357A binary capillary pump, G1389A autosampler, G1315B photodiode array detector, and a G1316A column oven all running under Applied Biosystems Analyst 2.0 (build 1446) LC-MS software. The MS was calibrated at least once daily with a standard calibration mixture recommended by Applied Biosystems and the signal detection was optimised as needed. The data were acquired in the MOF MS negative mode. The MS parameters were as follows: accumulation time 1 s, mass range 400–2000 daltons, source gas 1–50 units, source gas 2–35 units, curtain gas 20 units, ion spray voltage was 4200 V, source heater 250 °C, declustering potential 80, focusing potential 265, declustering potential 2–15, ion release delay 6, ion release width 5. The column used was an Inertsil ODS-3 reverse phase C-18 column (3 μM, 150 × 3 mm, with a Metaguard column, from Varian). The initial HPLC conditions were
60% acetonitrile and 40% 0.25% formic acid in water, at a flow rate of 0.3 ml per min, and then to 80% acetonitrile over 60 min. The effluent was also monitored at 210 nm on the PDA.

2.6. Macrophages culture and cell viability assay and treatments

Macrophage cell line RAW 264.7 was cultured and cell viability assay was conducted using the CellTiter 96 Aqueous One Solution proliferation assay kit (Promega Corporation, Madison, WI, USA) as previously indicated (Puangpraphant & de Mejia, 2009). The cells were treated with different concentrations of matesapoin fractions (1–100 μM) for 24 h. The percentage of viable cells was calculated with respect to cells treated with 0.1% DMSO. Solvents used at these concentrations showed no cytotoxicity on cell viability (>80%). DMSO at <0.1% presented 95.8% cell viability. Cell proliferation assay was conducted using the CellTiter 96 Aqueous One Solution proliferation assay kit (Promega Corporation, Madison, WI, USA).

2.7. Nitrite, PGE2, iNOS and COX-2 measurements

After 24 h of treatment and LPS induction, culture supernatant was collected and measured to determine nitrite and PGE2. COX-2 enzyme concentration of matesaponins fractions (1, 10, 25, 50 μM) for 24 h. For CCD-33Co, 1 × 10^5 cells per well were seeded in a 6-well plate and cells were allowed to grow for 48 h at 37 °C in 5% CO2/95% air. After 48 h incubation, cells were treated with different concentrations of matesapoin fractions (1, 10, 25, 50 μM) and 1 μg/mL of LPS for 24 h. After 24 h treatment, the spent medium was collected and analysed for NO and PGE2. Cell lysates were used to study the effect of matesapoin fractions on the expressions of COX-2 and iNOS.

2.8. Western blot of NF-κB p50 and p65 protein expression

NF-κB p50 and p65 expressions were determined in the cell cytoplasm and nucleus as previously discussed (Puangpraphant & de Mejia, 2009). Briefly, RAW 264.7 macrophages were treated 24 h with different concentrations of matesapoin fraction F (1, 10, 25, 50 μM) and 1 μg/mL of LPS. Nuclear and cytoplasmic proteins were isolated with a buffer extraction and centrifugation system NE-PER® (Pierce Biotechnology, IL) according to the manufacturer's recommendations.

2.9. HT-29 and CCD-33Co culture and cell proliferation assay

HT-29 cells were cultured in growth medium containing McCoy 5A, 1% penicillin/streptomycin, 1% sodium pyruvate and 10% fetal bovine serum at 37 °C in 5% CO2/95% air. CCD-33Co colon fibroblasts were cultured in Eagle’s Minimum Essential Medium containing 10% FBS and 1% penicillin/streptomycin. Cell proliferation was determined using CellTiter 96 Aqueous assay kit (Promega, Madison, WI) as previously indicated (Puangpraphant & de Mejia, 2009). For CCD-33Co, 1 × 10^5 cells per well were seeded in a 96-well plate and allowed to grow to confluency for one week with replacement of the medium every other day. For HT-29, 5 × 10^4 cells per well were seeded in a 96-well plate and total volume was adjusted to 200 μl with growth medium and allowed to grow for 24 h. Both cells were then treated with different concentrations of matesapoin fraction F (1, 10, 25, 50 μM) for 24 h.

2.10. Analysis of Bax and Bcl-2 expression and caspase-3 activity in HT-29 cells

HT-29 cells were seeded at a density of 2 × 10^5 cells per well in a six-well plate for 24 h at 37 °C in 5% CO2/95% air. After 24 h incubation, cells were treated with matesapoin fraction F (1 to 50 μM) for 24 h. After treatment, cells were trypsinised and suspended in lysis buffer composed of 62.5 mM Tris–HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue, 5% β-mercaptoethanol and protease inhibitor cocktail (Thermo Scientific, Rockford, IL). Cell suspension was then used for Western blot for actin, Bax and Bcl-2 using antibodies (1:200).

Caspase-3 activity was measured using a fluorescence assay kit (Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's protocol. HT-29 cells were seeded in a 96-well plate in 100 μl of culture medium at a density of 5 × 10^4 cells/well and incubated at 37 °C in a CO2 cell culture incubator overnight. Cells were treated with matesapoin fraction F, quercetin and cisplatin (1–50 μM) for 24 h in a 37 °C incubator. Relative fluorescent intensity was then measured with a plate reader FLx800thi (Biotek, Winooski, VT) (excitation = 485 nm; emission = 535 nm).

2.11. Statistical analysis

Data are presented as means ± SD for the indicated number of independently performed experiments. Data were analysed using one-way ANOVA and means were considered to be significantly different at p < 0.05 as determined by least significant differences (LSD).

3. Results and discussion

3.1. Identification of saponins extracted from mate dry leaves

We obtained six fractions (A, B, C, D, E, F) of complex mixtures that absorbed UV light at 210 nm. The presence of saponins was confirmed in only fractions D, E, and F. Table 1 lists the identified saponins, their retention times (RT), major mass ions, fragment ions, name and concentration. Fig. 1 shows LC-MS of an analytical run of matesapoin fraction F. Under these chromatographic and mass analysis conditions, mate saponins form adducts with formic acid, giving a characteristic mass identification pattern of [M-1]- and [M + 46]-. It appears to be two isomeric forms of matesapoin 2, which is consistent with the observations of Martinet et al. (2001). Saponins in Yerba mate leave extracts gave a total concentration of 11.7 ± 0.37 mg/g dry weight (dw) (matesapoin 1, 5.04 ± 0.16 mg/g dw; matesapin 2a, 3.55 ± 0.11 mg/g dw; matesapin 2b, 3.08 ± 0.09 mg/g dw). Matesapions 3, 4, and 5 were found only in trace amounts.

Yerba mate leaves were shown to contain 1.2 g of saponins per 100 g of dry leaves, mostly in the form of matesapins 1 and 2. It is important to note that saponins are not as soluble in water as they are in organic solvents, so the levels of extractable saponins in mate teas will be much lower than what is actually present in the leaves. Two recent reports (Borré et al., 2010; Coelho, Gnoatto, Bassani, & Schenkel, 2010) have provided information on the levels of saponins in mate leaves and plant tissues. Borré et al. (2010) have reported an estimate of 45 mg/g (4.5%) total saponin content in leaves based on an aqueous ethanol extraction and a summation of absorbing peaks at 210 nm. This method appears to overestimate the levels of saponins in the tissues as the ten individual peaks being summed have not been confirmed by the authors to be saponins. In contrast, Coelho et al. (2010) have determined a range of 0.3 to 1 mg/g (0.1%) based on the determination of matesapins 1, 2, and 3 in hot aqueous extracts of mate leaves. Comparison of our results indicates that the tea preparation may extract about 10% of the available saponins.

3.2. Effect of matesapoin fractions on NO, PGE2 production and iNOS, COX-2 expression in LPS-induced RAW 264.7 cells

Macrophages showed a survival rate of >90% when incubated with matesapoin fractions at a concentration ≤50 μM. Therefore,
in this study, a concentration of 1–50 μM was used to treat the cells and thus prevent the compounds from having cytotoxic effect.

Fractions A, B and C had no effect on NO production and did not reduce iNOS and COX-2 expressions (data not shown). This may be due to the absence of saponins and high content of sugars. Fig. 2 shows that fractions D and E did not significantly reduce NO production (Fig. 2A), but showed inhibition of iNOS expression in a dose-dependent manner (Fig. 2B). Fraction F significantly reduced NO production at 50 μM (Fig. 2A) and inhibited iNOS expression in a dose–dependent manner (Fig. 2B). Fraction D significantly inhibited COX-2 expression at 25 μM (Fig. 2C) and reduced PGE₂ production at 10 μM (Fig. 2D). Fraction E significantly reduced COX-2 expressions at 25 μM and PGE₂ production at 1 μM. While fraction F significantly reduced COX-2 expression in a dose–response manner starting at 10 μM and PGE₂ production at 10 μM. Table 2 presents the calculated concentration (based on the aglycone in μM) of matesaponins in fractions D, E, and F that resulted in a 35% reduction on pro-inflammatory parameters. Matesaponins in fraction D, E, and F inhibited COX-2/PGE₂ pathway. Fraction E and F seem to be more potent than fraction D in inhibiting pro-

Table 1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>TIC of TOF MS</th>
<th>Possible compound</th>
<th>Concentration (mg/g)</th>
<th>Total matesaponins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Retention Time (min)</td>
<td>[M+H]⁺ (m/z)</td>
<td>[M+HCOO]⁺ (m/z)</td>
<td></td>
</tr>
<tr>
<td>Fraction D</td>
<td>11.0</td>
<td>515</td>
<td>537</td>
<td>Dicaffeoylquinic acid</td>
</tr>
<tr>
<td></td>
<td>56.2</td>
<td>911</td>
<td>958</td>
<td>Matesaponin 1</td>
</tr>
<tr>
<td></td>
<td>55.3</td>
<td>1057</td>
<td>1104</td>
<td>Matesaponin 2a</td>
</tr>
<tr>
<td></td>
<td>60.4</td>
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<td>Matesaponin 2b</td>
</tr>
<tr>
<td></td>
<td>60.2</td>
<td>1219</td>
<td></td>
<td>Matesaponin 3</td>
</tr>
<tr>
<td></td>
<td>60.3</td>
<td>1383</td>
<td></td>
<td>Matesaponin 4</td>
</tr>
<tr>
<td>Fraction E</td>
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<td>911</td>
<td></td>
<td>Matesaponin 5</td>
</tr>
<tr>
<td></td>
<td>55.3</td>
<td>1057</td>
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<td>Matesaponin 1</td>
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<td>60.4</td>
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<tr>
<td></td>
<td>60.3</td>
<td>1383</td>
<td></td>
<td>Matesaponin 3</td>
</tr>
<tr>
<td>Fraction F</td>
<td>56.3</td>
<td>911</td>
<td>958</td>
<td>Matesaponin 1</td>
</tr>
<tr>
<td></td>
<td>55.4</td>
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<td></td>
<td>60.2</td>
<td>1219</td>
<td></td>
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<td>53.5</td>
<td>1382</td>
<td></td>
<td>Matesaponin 4</td>
</tr>
</tbody>
</table>

Fig. 1. LC-MS of an analytical run of matesaponins fraction F. The traces are fragment ions for m/z values of 947, 957, 1093 and 1103, respectively.
inflammatory markers. This may be due to different chemical composition in fraction D, which contains dicaffeoylquinic acids (Table 1). Fraction F showed more potent anti-inflammation activity than fraction E even though the latter had a higher saponin concentration.

Excessive production of NO, especially in macrophages, can lead to cytotoxicity, inflammation, carcinogenicity, and autoimmune disorders. Therefore, modulation of NO and iNOS could prevent inflammatory diseases. In this study, matesaponins fraction F was found to significantly suppress LPS-induced NO production in RAW 264.7 cells, due to its ability to inhibit the expression of iNOS. Inhibition of the pro-inflammatory cytokines production is believed to be one of the pathways by which matesaponins exert their anti-inflammatory effect (Puangpraphant and de Mejia, 2009).

The interaction between NO signalling and COX-2 is well documented and is a significant contributor to chronic inflammation which leads to carcinogenesis (Fukumura et al., 2006). It has been shown that a combination of COX-2 and NO inhibitors enhances the effect of chemoprevention of colon carcinogenesis (Rao et al., 2002). Matesaponins fractions D, E, and F inhibited the inducible enzymes, iNOS and COX-2, suggesting that saponins may have additional chemopreventive potential. It has been reported that saponins suppress the expression of COX-2 and iNOS, resulting in a marked lowering of PGE2 levels (Kim et al., 2006), the assumed precursor for formation and growth of malignant tumours. Manipulation of PGE2 pathways, and their impact on cancer growth and treatment, have become an important aspect of cancer prevention research (Hull, 2008).

3.3. Effect of matesaponin fraction F on NF-κB nuclear translocation by LPS-induced RAW 264.7 cells

Fraction F reduced nucleus protein expressions of NF-κB subunits p65 (Fig. 3A) and p50 (Fig. 3B) at a concentration of 25 μM, and showed no change expression in cytoplasm. Fraction F was

**Table 2**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>NO**</th>
<th>iNOS**</th>
<th>PGE2**</th>
<th>COX-2-**</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>&gt;50</td>
<td>36.3 ± 3.1^a</td>
<td>23.1 ± 4.8^a</td>
<td>45.7 ± 9.5^a</td>
</tr>
<tr>
<td>E</td>
<td>&gt;50</td>
<td>29.5 ± 2.9^b</td>
<td>22.3 ± 3.8^a</td>
<td>32.4 ± 7.1^a</td>
</tr>
<tr>
<td>F</td>
<td>&gt;50</td>
<td>43.7 ± 2.4^a</td>
<td>11.7 ± 1.5^b</td>
<td>17.0 ± 1.9^b</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences, p < 0.05.

IC50 is the concentration (μM) that resulted in 35% reduction of production/expression of pro-inflammatory responses (mean ± SD).

Abbreviations: NO, nitric oxide; iNOS, inducible nitric oxide synthase; PGE2, prostaglandin E2; COX-2, cyclooxygenase-2.

![Figure 2](image_url)
NF-κB is known to play a critical role in the regulation of cell survival genes and to induce the expression of inflammatory enzymes and cytokines, such as iNOS, COX-2, TNF-α, IL-1β, and IL-6 (Yoshimura, 2006). Blocking the NF-κB transcriptional activity in the macrophage nucleus can suppress the expression of iNOS, COX-2, and pro-inflammatory cytokines. A number of saponins have been studied in vitro and in vivo to clarify their effects on inflammation (Ahn et al., 2005; Cheeke, Piacente, & Oleszek, 2006; Choi et al., 2005; Kim et al., 2006; Leung et al., 2005; Matsui et al., 2009; Suh et al., 2007; Sur, Chaudhuri, Vedarismoni, Gomes, & Ganguly, 2001; Wang et al., 2008). Taken together, our results suggest that saponins from mate dry leaves may be beneficial in preventing inflammation and thus potentially reducing colon cancer. The molecular mechanisms are considered to be closely related to the inhibition of iNOS and COX-2 protein expression and through inhibition of NF-κB nuclear translocation.

3.4. Effect of matesaponin fraction F on cell proliferation of HT-29 colon cancer cells

Fig. 4 shows that matesaponins in fraction F caused a dose-dependent inhibition of HT-29 colon cancer cells proliferation. At a concentration of 50 μM, matesaponins in fraction F inhibited 12.7% of viable HT-29 cells when compared to cells not treated with matesaponins. At the highest concentration tested (100 μM), matesaponins caused a 48.6% inhibition. Matesaponins did not cause any cytotoxicity to CCD-33co normal colon fibroblasts up to 100 μM (data not shown).

Several studies have shown that saponins in plants are associated with a reduced risk of colorectal cancer by inducing cytotoxicity and apoptosis (Kim et al., 2008; Rao & Sung, 1995; Xiao, Huang, Zhu, Ren, & Zhang, 2007). Matesaponins have an ursolic acid aglycone; it has been shown that ursolic acid has strong anti-proliferative and apoptotic effects on HT-29 cells (Anderson, Liu, Nilsson, & Duan, 2003). We examined the anti-proliferative effect of matesaponins fraction F on human colon cancer HT-29 cells at various concentrations (1–100 μM). Matesaponins fraction F significantly decreased the proliferation of HT-29 cells by 48.6% at 100 μM. This inhibition concentration of matesaponins to HT-29 proliferation is closely to quercetin which showed IC_{50} to HT-29 at 81 μM. Thus, matesaponins fraction F could be a potential anti-cancer agent in colon cancer therapy. It is known that the bioavailability of saponins from various plant sources is low (1–4%) because of their poor oral absorption and gastrointestinal metabolism (Cai et al. 2008; Han, Sha, Wu, & Fang 2005; Hu, Reddy, Hendrich, & Murphy 2004; Komoto et al. 2010; Xu, Fang, & Chen 2003). However, saponins could be hydrolysed in the intestinal tract and the aglycone, ursolic and/or oleanolic acids, go through the colon. Ursolic and oleanolic acids have been shown to have a protective effect against colon carcinogenesis in vivo (Furtado et al. 2008). After oral intake of saponins (100 mg/kg body weight), the concentration in plasma of mice was 1063 ng/ml after 0.5 h (Komoto et al. 2010). Also, eight healthy women ingested 434 μmol soyasaponin, and its metabolite was found (36.3 μmol) in a 5-d faecal sample (Hu et al., 2004). They also showed no cytotoxic effect of saponin on Caco-2 cells at concentrations up to 3 mM.

3.5. Effect of matesaponin fraction F on Bcl-2/BAX protein expression and caspase-3 activity in HT-29 colon cancer cells

Fig. 5 shows that matesaponin fraction F significantly inhibited the expression of Bcl-2 at 100 μM (Fig. 5A), and significantly increased the expression of Bax at 25 μM (Fig. 5B).

Fig. 5C shows that the activity of the major apoptotic factor caspase-3 was increased significantly with matesaponin fraction.
Caspase-3 activity was elevated in HT-29 cells by matesaponins fraction F treatment in a similar dose-dependent manner as quercetin. To investigate the mechanism of action of these results, we analysed the apoptosis induction of HT-29 cells treated with matesaponins fraction F compared with quercetin and cisplatin, by assaying the protein expression of apoptosis mediators. Members of the Bcl-2 family of proteins are critical regulators of the apoptotic pathway, controlling mitochondrial permeability and cytochrome c expression (Oakes, Lin, & Bassik, 2006; van Delft & Huang, 2006). These proteins consist of the major anti-apoptotic proteins, Bcl-x(L) and Bcl-2, and the major pro-apoptotic proteins Bax and Bak. Following exposure to matesaponins fraction F, Bax expression increased while Bcl-2 expression decreased. Bax controls mitochondrial permeability and cytochrome c expression. The release of cytochrome c from mitochondria to the cytoplasm is a key step in the initiation of apoptosis. As a downstream product of cytochrome c, caspases are critical mediators of the principle factors found in apoptotic cells. Among them, caspase-3 is a frequently activated death protease, catalysing the specific cleavage of many key cellular proteins (Abu-Qare & Abou-Donia, 2001). In the present study, matesaponins fraction F induced the activity of caspase-3 at 50 μM, similar to quercetin and cisplatin. Thus, matesaponins inhibited colon cancer cells proliferation by inducing apoptosis through activating caspase-3 activity. These could contribute to cancer chemotherapy and the inhibition of tumour growth.

4. Conclusions

In the current study we found mate leaves contained 12 mg/g (1.2%) extractable saponins mostly in the form of matesaponins 1 and 2. The matesaponins processed anti-inflammatory activity through NF-κB pathways. Our results also showed that matesaponins inhibit colon cancer cell (HT-29) proliferation. Matesaponin-containing fraction F activates a specific intracellular apoptosis pathway in HT-29 cells. This saponin fraction also increased the expression of the pro-apoptotic protein Bax, decreased the expression of anti-apoptotic protein Bcl-2; and subsequently activated caspase-3. These findings suggest that apoptosis induction in matesaponins-treated HT-29 cells could be associated with a caspase-dependent cascade that involves the activation of the mitochondrial pathway, initiated by the inhibition of Bcl-2 and the activation of Bax. Our findings suggest the possible value of matesaponin fraction F against human colon cancer by promoting apoptosis of cancer cells.

Acknowledgements

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