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Phytochemical screening, characterization, and in vitro anti-metastatic effects of the methanolic leaf extract of *Momordica cardiospermoides* in MDA-MB 321 cells

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ABSTRACT

Introduction: *Momordica cardiospermoides* represents an inexhaustible source of natural products. We examined the acetone and methanolic extracts of *M. cardiospermoides* leaves' phytochemical composition, antioxidant activities, and anti-metastatic properties in MDA-MB-231 cells.

Methods: The aluminium chloride and Folin-Ciocalteu methods were employed to determine the extracts' phytochemical contents. Assessment of antioxidant activities employed the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The extracts were further characterized with ultra-high-performance liquid chromatography (UHPLC). The cell count and viability kit were utilized to assess viability in HEK-293 and MDA-MB-231 cells. The occurrence of cell death was determined by the annexin V and dead cell assay. The methanolic extract was assessed against cell adhesion and migration using the cell-extracellular matrix and wound healing assays. The effects of the methanolic extract on tissue inhibitors, including metalloproteinase-1 (TIMP-1), matrix metalloproteinases-2 (MMP-2), and -9 (MMP-9) were determined by the human angiogenesis antibody array kit and Western blot analysis.

Results: The acetone extract had higher antioxidant activity and total contents than the methanolic extract. UHPLC revealed abundant luteolin, luteoloside, quercetin, stearidonic acid, and salicylamide in the acetone extract, and luteolin, astragalin, and trigonelline in the methanolic extract. The methanolic extract was selectively cytotoxic towards MDA-MB-231 cells while the acetone extract was significantly cytotoxic in both cell lines. Apoptosis was induced by the methanolic extract and the acetone extract induced significant necrosis. The methanolic extract suppressed migration by significantly inhibiting wound closure and inhibited cell adhesion. MMP-2 and MMP-9 were significantly downregulated and TIMP-1 was upregulated.

Conclusion: The methanolic extract *M. cardiospermoides* possesses anti-metastatic activity.

Implication for health policy/practice/research/medical education:
The study gives insight into the phytochemistry of the extracts prepared from the leaves of Momordica cardiospermoides. Additionally, the study provides novel findings on the *in vitro* anticancer and anti-metastatic potential of *M. cardiospermoides* extracts in breast MDA-MB-231 cancer cells, which can help in the preparation of new drugs.

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Introduction

As per the World Health Organization (WHO) report of 2020, breast cancer is the most frequently diagnosed cancer type, with incidence and mortality rates of 13.1 and 8.2%, respectively (1). Effective migrastatic agents should not rely solely on primary tumour responses such as tumour shrinkage. These agents exert their effects on cancer cells by inhibiting at least one or more of the processes involved in the multistep metastatic cascade. The search for novel target-specific migrastatic agents, that exert little to no side effects and interfere with processes such as cell migration and invasion, continues (2,3).

Cancer metastasis requires extracellular matrix (ECM) remodeling by a proteolytic breakdown. Matrix metalloproteinases-2 and -9 are two proteinases primarily involved in ECM remodeling and basement membrane degradation (4). When proteolytic degradation of the ECM occurs, cancer cells lose adhesion receptors that

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mediate cell-extracellular matrix adhesion and transduce signals that regulate numerous aspects of cell behavior specifically proliferation, migration, and reattachment at the secondary site. Moreover, during cell migration, cells generate active pulling forces which are transmitted through focal adhesion complexes to ECM fibers, remodel the ECM and propagate to the desired anatomical destination (5,6). As evidenced *in vitro* and *in vivo,* tissue inhibitors of metalloproteinases (TIMPs) successfully regulate matrix metalloproteinase (MMP) activity. TIMP-1 downregulation contributes to increased invasiveness, while its increased expression controls MMP activity resulting in the maintenance of the ECM integrity (7), thus inhibiting the dissemination of metastatic tumor cells.

For decades, scientists have used herbal remedies to guide them in their pursuit of novel medications. This has yielded the discovery of a spectrum of therapeutic properties such as antioxidant, anti-inflammatory, and more. *Momordica cardiospermoides* is an herbaceous climber that is harvested for its nutritional value and is used in orthodox medicine to treat numerous ailments, including conditions related to diabetes, inflammatory diseases, and cancer (8). Recently, Ramabulana et al (9) investigated the metabolomics of four *Momordica* species (*charantia, balsamina, foetida*, and *cardiospermoides*) and concluded that *M. cardiospermoides* could be an alternate source of metabolites as it contains phytoconstituents that are not found in the other *Momordica* plant species investigated. This study, therefore, investigated the methanolic and acetone extracts of *M. cardiospermoides* leaves' phytochemical composition and their potential to interfere with cell migration, adhesion, and invasiveness in MDA-MB-231 cells.

Materials and Methods

Materials

Methanol, acetone, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picryhydrazyl (DPPH) solution, sodium carbonate, folin-ciocalteau, gallic acid, potassium acetate, aluminium chloride, quercetin, and tannic acid were purchased from Rochelle Chemicals (RSA). Dulbecco's Modified Eagle Medium/DMEM was from HyClone Laboratories (USA). Whatman no. 2 filter paper and curcumin were purchased from Sigma-Adrich. Cell lines were procured from the American Type Culture Collection (USA). Fetal bovine serum (FBS) was obtained from Gibco Life Technologies (USA). The Count and viability kit, Annexin V and dead cell kit, and the Muse® cell analyzer are from Merck (Germany). BCA protein quantification kit and PVDF membranes were procured from Thermofisher Scientific (Rockford, USA). Antibodies: Goat anti-mouse IgG HRP-conjugated, mouse anti-MMP-2, mouse anti-β-actin, and mouse anti-MMP-9 were all obtained from Novus Biologicals (USA). The Human angiogenesis antibody array kit was purchased from R&D Systems (USA). Trans-blotter used was obtained from Bio-Rad (USA). The phase-contrast inverted microscope used was from Nikon (Japan).

Methods

Plant harvesting and extraction

Fresh leaves of *M. cardiospermoides* were harvested in Polokwane, South Africa. Identification and authentication of fresh leaves was done by Dr B.A. Egan, one of the corresponding curators at the Larry Leach Herbarium (UNIN). A voucher specimen (UNIN 1220084) was deposited there. Dried leaves were ground to a fine powder. Exhaustive extraction of 20 g of powder in 200 mL of absolute acetone or methanol was done. The extracts were filtered and the filtrates dried under an industrial fan. The dried extract residues were redissolved in their respective extractants to stocks of 10 mg/mL solutions for phytochemical tests. A stock solution of 100 mg/mL was prepared in 100% dimethyl sulfoxide (DMSO).

Phytochemical analysis

Analysis of antioxidant activity

The antioxidant activities of *M. cardisopermoides* methanolic (MCM) and *M. cardiospermoides* acetone (MCA) extracts were determined as described by Phuyal *et al.* (10). The MCA or MCM extracts (1 mg/mL) or L-ascorbic acid (1 mg/mL) were diluted to various concentrations (0 - 100 µg/mL). The extracts or L-ascorbic acid were incubated with DPPH solution (200 µM) for 30 minutes in complete darkness. Absorbances were read at 515 nm. The extracts' inhibitory effects were calculated using the formula below:

$$\%Inhibition = \frac{(Absorbance\,of\,control - Absorbance\,of\,sample)}{Absorbance\,of\,control} \times 100$$

Total phenolic content

The extracts' phenolic contents were determined as described by Phuyal et al (10) with modifications. Folin-Ciocalteu phenol reagent (250 µL of 10% (v/v)) was added to the MCM or MCA extract (66.6 µL), shaken, and incubated for 5 minutes in the dark. Next, sodium carbonate (Na_2CO_3) solution (250 µL of 7% (w/v)) was added and incubated for 90 minutes. Gallic acid prepared in methanol (0–0.5 mg/mL) was the standard. Absorbances were measured at 750 nm. The extracts' TPC was calculated according to the formula below and the data were presented as mg of gallic acid equivalent per gram of extract (GAE mg/g).

$$TPC = \frac{M}{V} \times C$$

Where 'C' is the concentration of gallic acid (mg/mL); 'V' is the volume of the plant extract; and 'M' is the weight of the extract in grams.

Total tannin content

The extracts' tannin contents were determined as described by Haile and Kang (11) with modifications. Folin-Ciocalteau phenol reagent (100 µL of 10% (v/v)), sodium carbonate (Na_2CO_3) solution (1000 µL of 35% (v/v)), distilled water (1500 µL), and MCA or MCM extract (20 µL) were incubated together for 30 minutes. Tannic acid (0–0.5 mg/mL) in acetone was the standard. Absorbances were measured at 725 nm. The extracts' TTC was calculated according to the formula below and the data were presented as mg of tannic acid equivalent per gram of extract (TAE mg/g).

$$TTC = \frac{M}{V} \times C$$

where 'C' is the concentration of tannic acid (mg/mL); 'V' is the volume of the plant extract; and 'M' is the weight of the extract in grams.

Total flavonoid content

The extracts' flavonoid contents were determined as described by Phuyal et al (10). Distilled water (1400 µL), MCA or MCM extract (50 µL), and 1 M potassium acetate (50 µL) were incubated together for 30 minutes. Quercetin in methanol (0 – 0.5 mg/mL) was the standard. Absorbances were measured at 420 nm. The extracts' TFC was calculated according to the formula below and the data were presented as mg of quercetin per gram of extract (QE mg/g).

$$TFC = \frac{M}{V} \times C$$

where 'C' is the concentration of quercetin (mg/mL); 'V' is the volume of the plant extract; and 'M' is the weight of the extract in grams.

Ultra-high-performance liquid chromatography

A UHPLC/SCIEX X500 QTOF system was used to separate the extracts as described by Du et al (12). The extracts' components were separated using a C18 column mobile phase with acetonitrile in addition to 0.1% formic acid in water and a 0.3 ml/min flow rate. Samples as well as standard solutions had a 10 µL injection volume. LC-MS analysis was performed in positive and negative ESI mode. The IS voltage and ion source temperature were set to 5500 V and 550 °C, respectively. SWATH acquisition method was performed in positive electrospray ionisation mode. MS/MS fragmentation was attained at 35 V to ±15 V spread collision energy and de-clustering potential of 80 V. To achieve MS/MS coverage fully, the dynamic background subtraction was activated. MS/MS data was acquired without an inclusion list. The SCIEX OS software version 1.7.1. was used to process the data.

Cell cultures and treatments

MDA-MB-231 and HEK-293 cells were grown in DMEM supplemented with a 10% (v/v) FBS (heat-inactivated). They were maintained at 37 °C with a 5% CO_2 atmosphere. Stock solutions of the extracts were diluted to experimental concentrations using DMEM. Untreated cells were the negative control and 25 µM curcumin-treated cells were positive control.

In vitro analysis

Cell viability assay

The assessment of viability was done using the cell count and viability kit. Cells (5×10^3/well) were seeded and exposed to curcumin or the MCA or MCM extracts (0–300 µg/mL) for 24 hours. Results were analysed by the muse cell analyzer. Data were presented as the percentage (%) of viable cells.

Analysis of dying cells

The occurrence of death in cells was determined with the annexin V and dead cell assay. MDA-MB-231 cells (3×10^5/well) were seeded in a 24-well plate. They were treated for 6 or 24 hours with the MCA or MCM extract (0–300 µg/mL). Results were analysed by the muse cell analyzer. Data were presented as the percentage (%) of cells.

Cell migration assay

The wound healing/scratch assay described by Mabasa et al (13) was used to determine the effects of the MCM extract on cell migration. MDA-MB-231 cells were grown in a 6-well plate to form a monolayer. A 200 µL pipette tip was used to wound cells. They were then treated for 6 and 24 hours with the MCM extract (150 and 300 µg/mL) or with curcumin. An inverted microscope was used to monitor and capture photographs of wound sizes under 4x magnification. The sizes of wounds were measured with the LC-micro software (Version: 510). Data were expressed as the percentage wound closure calculated according to the following formula:

$$Wound\ closure\,(\%) = \frac{Wound\ size\,(T0) - Wound\ size\,(Tn)}{Wound\ size\,(Tn)} \times 100$$

Cell attachment assay

Cell-ECM adhesion assay described by Mabasa et al (13) was employed in assessing the effects of the MCM extract on MDA-MB-231 cells' ability to re-attach to a cell culture plate. Cells (3×10^6/well) were seeded in a 24-well plate. They were then treated for 24 hours with the MCM extract (150 or 300 µg/mL) or curcumin. The attached cells were captured under the 10x objective of an inverted microscope after staining with crystal violet [0.1% (w/v)]. DMSO was used to redissolve the stain cells and the absorbance readings were measured at 565 nm. The percentage of attached cells was calculated using the formula below and the data were presented as cell adhesion (%).

$$Cell\ adhesion\,(\%) = \frac{Absorbance\ of\ treated}{Absorbance\ of\ untreated\ cell} \times 100$$

Biochemical analysis

Western blot analysis

The assessment of MMP-9 and MMP-2 protein expression was achieved through Western blot analysis, described by Lee et al (14). Proteins were extracted using 250 µL chilled RIPA lysis buffer. The BCA protein quantification kit was employed to quantify proteins. Aliquots containing 30 µg cellular proteins were resolved on sodium dodecyl-sulphate polyacrylamide (SDS-PAGE) gels (8–12%) and electroblotted onto PVDF membranes. Membranes were then blocked with 0.05% TBS-Tween in 5% (w/v) fat-free milk. After washing, the membranes were incubated with mouse antibodies against MMP-9 (1:500), mouse MMP-2 (1:500), and β-actin (1:1000) overnight. They were then incubated for 2 hours with goat anti-mouse IgG HRP-conjugated antibody. Next, membranes were incubated for 5 minutes with a chemiluminescent reagent mix and photographed using the C-DiGit blot scanner. Data were expressed as the percentage pixel density of each band.

Human angiogenesis antibody array

The assessment of TIMP-1 expression made use of the Human angiogenesis antibody array kit. Cell lysis was accomplished by using a RIPA buffer. The BCA protein quantification kit was employed to quantify proteins. Membranes were blocked for an hour using 2 mL of array buffer 7. Samples of 300 µg proteins were incubated (1 hour) with 15 µL of a detection antibody cocktail and array buffers 4 and 5. Membranes were maintained at 4 °C overnight with the protein/antibody cocktail. An hour incubation with streptavidin-HRP was proceeded by a 5-minute incubation with a chemiluminescent reagent mix and captured using a C-DiGit blot scanner. Data were expressed as the percentage pixel density of each spot.

Statistical analysis

Treatments and controls were considered statistically different as calculated on the GraphPad Instat3 using ANOVA (one-way) and Dunnett's comparison tests. Data were expressed as mean ± standard deviation (SD). $P \leq 0.05$ was considered significant.

Results

The phytochemical profiles of *Momordica cardiospermoides* extracts

The MCM extract possessed metabolites 1–3 (Table 1) identified as trigonelline ($C_7H_7NO_2$) luteolin ($C_{15}H_{10}O_7$), and astragalin ($C_{12}H_{20}O_{11}$). Comparatively, the MCA extract possessed metabolites 4–8 identified as stearidonic acid ($C_{18}H_{28}O_2$), quercetin ($C_{15}H_{10}O_7$), luteoloside ($C_{21}H_{20}O_{11}$), luteolin ($C_{15}H_{10}O_7$), and salicylamide ($C_7H_7NO_2$) (Figure 1).

Free radical scavenging potential/activity and total contents of *Momordica cardiospermoide*s extracts

The MCA extract had the highest free radical scavenging activity (Figure 2a), and higher total contents (32.6933 QE mg/g, 23.0683 GAE mg/g, and 22.0442 TAE mg/g) comparatively to the MCM extract (7.692 QE mg/g, 14.2349 GAE mg/g, and 11.3164 TAE mg/g) (Figure 2b).

The effects of *Momordica cardiospermoides* extracts on HEK-293 and MDA-MB-231 viability

The MCM extract significantly decreased MDA-MB-231 (Figure 3a) viability (concentration-dependent) without showing any toxicity in HEK-293 cells (Figure 3b). Comparatively, the MCA extract significantly ($P \leq 0.001$) decreased MDA-MB-231 (Figure 3c) and HEK-293 (Figure 3d) viability in a concentration-dependent manner. Moreover, only the MCM extract displayed selective cytotoxicity. Treatment with curcumin significantly decreased viability in HEK-293 cells.

Mode of MDA-MB-231 death induced by *Momordica cardiospermoides* extracts

The MCA extract significantly induced necrotic cell death preferably over apoptosis with increased concentration and incubation period (Figure 4a). In contrast, the MCM extract exhibited a time and concentration-dependent induction of apoptotic cell death (Figure 4b). Treatment with curcumin increased apoptosis induction with time.

Table 1. Compounds identified in the acetone or methanolic extracts of Momordica cardiospermoides using SCIEX X500 QTOF system

Observed mass (m/z)	Actual mass	Library score	Molecular formula	Metabolite found	Extract
138.0547	138.0547	92.6	$C_7H_7NO_2$	Trigonelline	Methanolic extract
287.0550	287.0550	99.7	$C_{15}H_{10}O_7$	Luteolin	Methanolic extract
449.1081	499.1081	85.6	$C_{12}H_{20}O_{11}$	Astragalin	Methanolic extract
277.2163	277.2163	63.6	$C_{18}H_{28}O_2$	Stearidonic acid	Acetone extract
303.0499	303.0499	70.1	$C_{15}H_{10}O_7$	Quercetin	Acetone extract
449.1081	449.1081	73.3	$C_{21}H_{20}O_{11}$	Luteoloside	Acetone extract
287.0550	287.0550	91.1	$C_{15}H_{10}O_7$	Luteolin	Acetone extract
138.0547	138.0547	63.3	$C_7H_7NO_2$	Salicylamide	Acetone extract

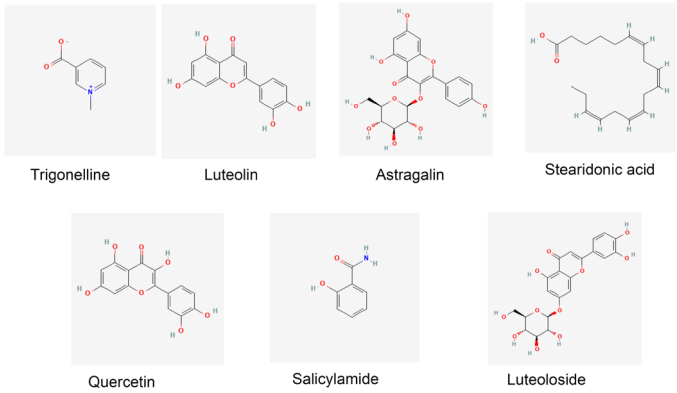

Figure 1. Structures of compounds identified in *Momordica cardiospermoides* acetone or methanolic extracts obtained from PubChem.

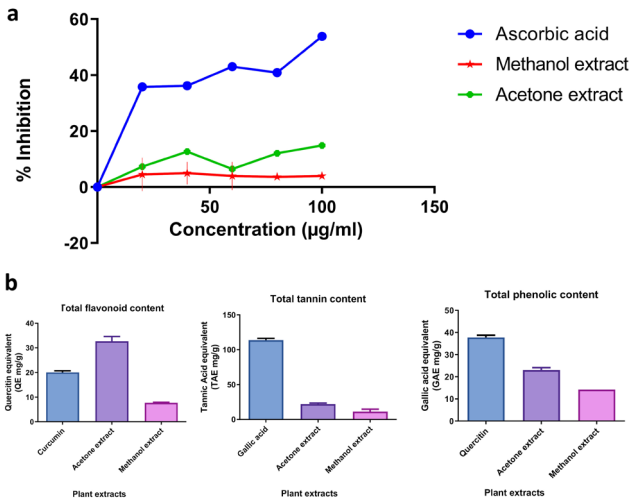

Figure 2. Analysis of the antioxidant activities (a) and total contents (b) of *Momordica cardiospermoides* acetone (MCA) and *Momordica cardiospermoides* methanolic (MCM) extracts. QE: quercetin equivalent; TAE: tannic acid equivalent and GAE: gallic acid equivalent.

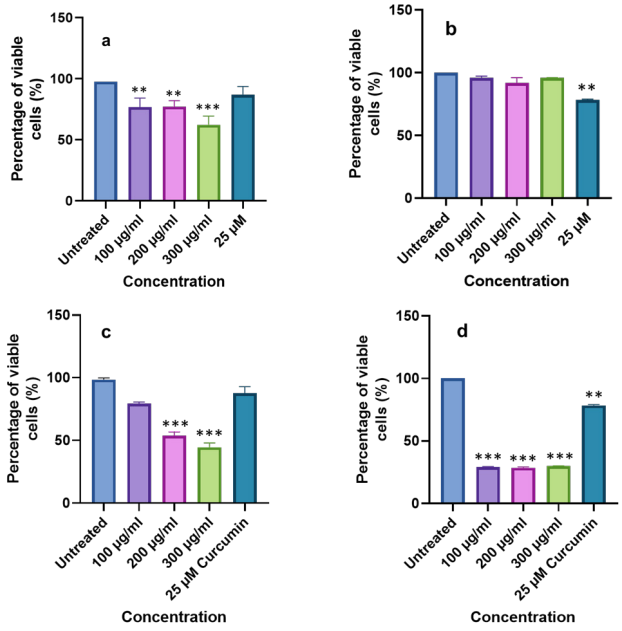

Figure 3. *Momordica cardiospermoides* methanolic (MCM) extract's effects in (a) MDA-MB-231 and (b) HEK-293 cells, and *Momordica cardiospermoides* acetone (MCA) extract in MDA-MB-231 (c) or HEK 293 (d). ** $P \leq 0.01$ and *** $P \leq 0.001$ vs untreated group.

Anti-migratory actions of the methanolic extract in MDA-MB-231 cells

The MCM extract inhibited cell migration at both 6- and 24-hour time points with an increase in concentrations (Figure 5a). Analysis of the wounds showed a significant time- and concentration-dependent decrease in wound closure (Figure 5b) compared to the untreated control. A similar effect was observed in cells treated with curcumin.

The methanolic extract's influences on protein expression

The MCM extract downregulated MMP-2 and MMP-9 (Figure 6a). The decrease in mean pixel density was significant for both (Figure 6b). Similarly, TIMP-1 expression was also decreased as the concentration of the extract increased (Figure 6c). Additionally, curcumin treatment significantly decreased the expression of all three.

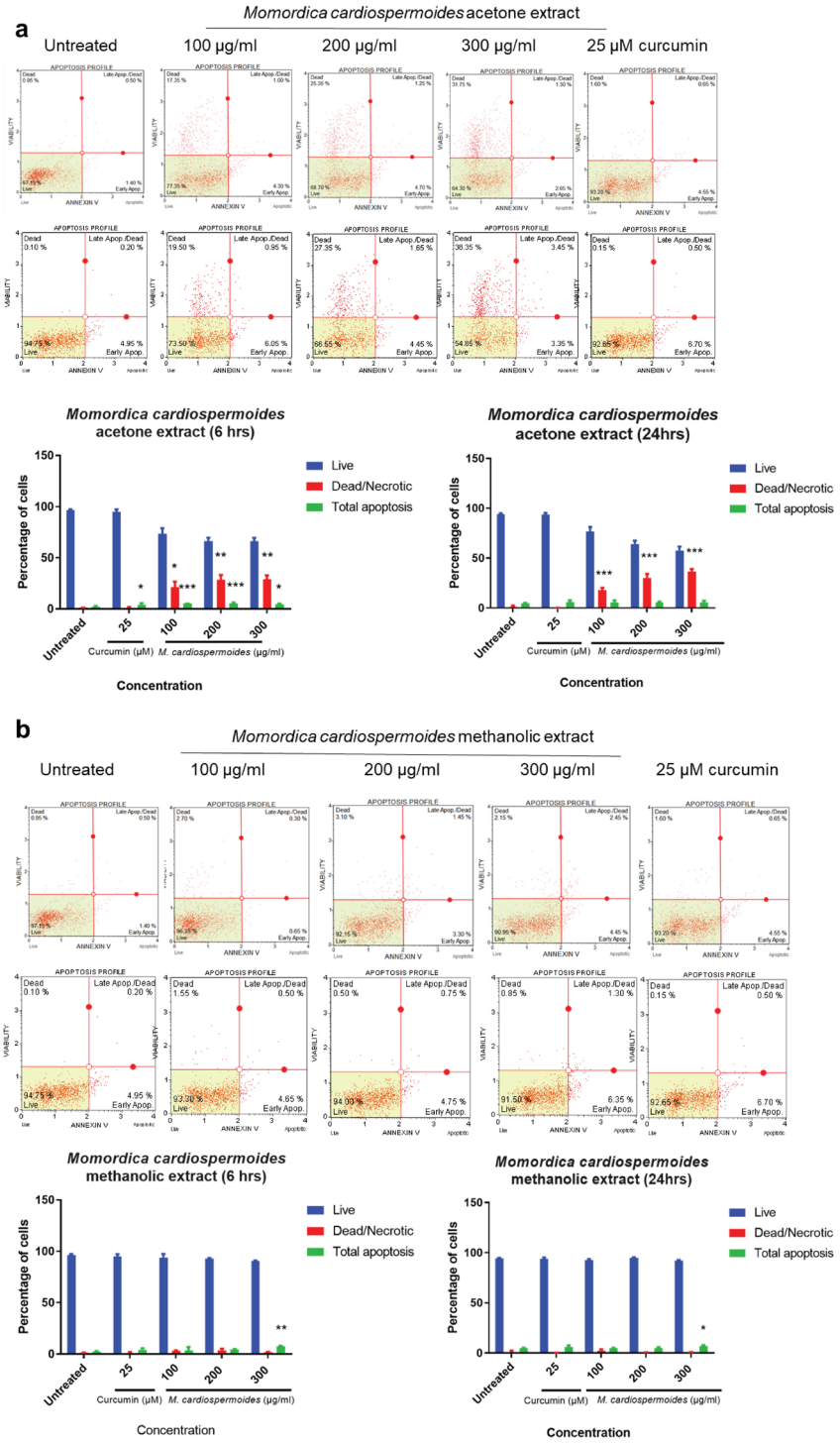

Figure 4. Mode of cell death induced in MDA-MB-231 cells by treatment with *Momordica cardiospermoides* acetone extract (a) or *M. cardiospermoides* methanolic extract (b). * $P \leq 0.1$, ** $P \leq 0.01$ and *** $P \leq 0.001$ vs untreated group.

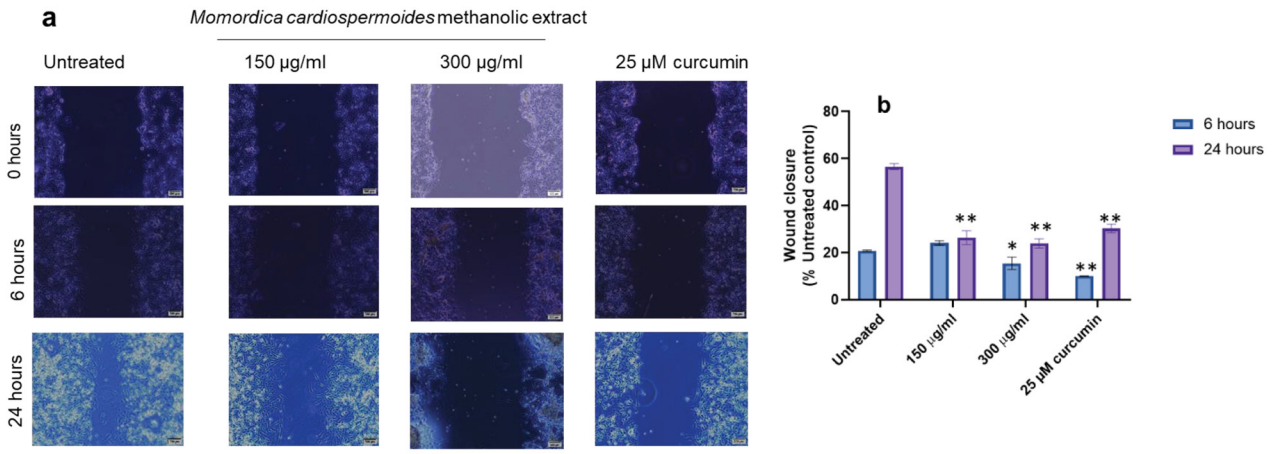

Figure 5. Effects of *Momordica cardiospermoides* methanolic (MCM) extract on cell migration. Photographs were captured using an inverted light microscope (a). Wound sizes were measured and expressed as percentage wound closure (b). *$P \leq 0.05$ and ** $P \leq 0.01$ vs untreated group.

The methanolic extract's effects on the adhesive properties of MDA-MB-231 cells

The MCM extract significantly decreased the attached cells compared to untreated cells (Figure 7a). Furthermore, the percentage of cell adhesion was inhibited in a concentration-dependent manner (Figure 7b). Moreover, treatment with the positive control significantly decreased the percentage of cell adhesion.

Discussion

Development of effective treatment strategies for metastatic cancers requires the inhibition of fundamental processes of the cascade, target-specificity, and the development of both pre-clinical and clinical approaches that have no reliance on primary tumor responses (3). *M. cardiospermoides* is traditionally used to treat various ailments and has been reported to produce distinct metabolites clustered in their own molecular family (9), especially when compared to the extensively studied *M. balsamina* and *M. charantia* species. As such, this study explored the antimetastatic potential of *M. cardiospermoides* extracts in MDA-MB-231 cells. The current study employed the UHPLC/SCIEX X500 QTOF system to identify the most dominant phytochemical metabolites in the MCM and MCA extracts of *M. cardiospermoides*. Metabolites astragalin, luteolin, and trigonelline were found to be abundant in the MCM extract. Trigonelline is an alkaloid that exerts its anti-cancer activities through the induction of apoptotic cell death, suppressing ROS-generating systems *in vitro,* and triggering G2 phase cell cycle arrest in several animal cells (15,16). The flavonoid luteolin possesses anti-inflammatory and anti-cancer activities related to apoptosis induction and redox regulation, thereby preventing cell proliferation and suppressing metastasis and angiogenesis (17). Astragalin, another flavonoid, reportedly mitigates inflammation and oxidation

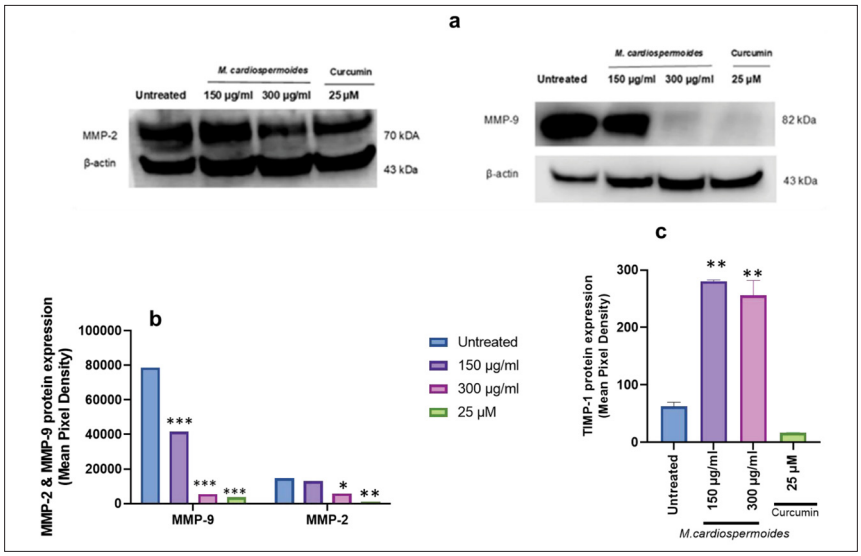

Figure 6. Effects of *Momordica cardiospermoides* methanolic (MCM) extract on matrix metalloproteinase-9/MMP-9 and matrix metalloproteinase-2/MMP-2 (a, b), and tissue inhibitor of metalloproteinase-1/TIMP-1 (c) expression. *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$ vs untreated group.

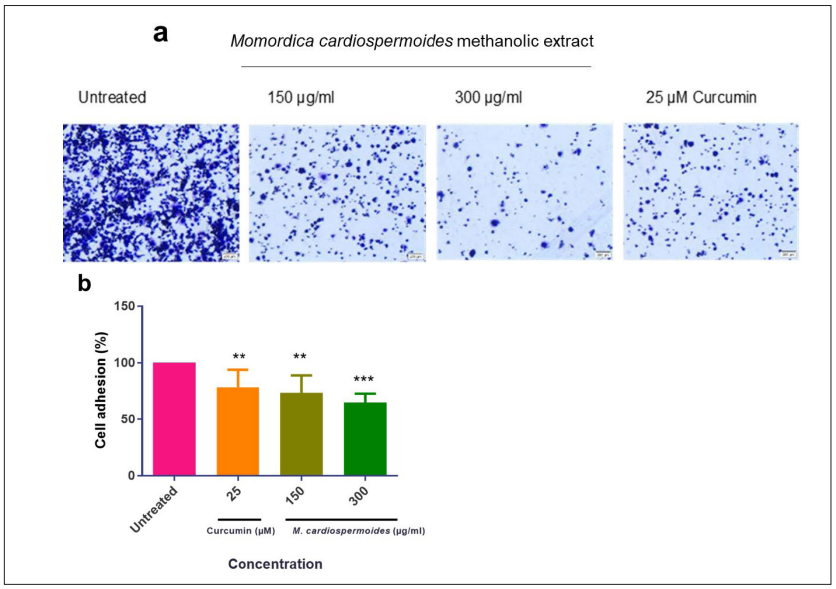

Figure 7. Effects of *Momordica cardiospermoides* methanolic extract on the cells' ability to re-adhere to a tissue culture plate. Photographs were captured using an inverted light microscope (a). Cells were counted and data were expressed as a percentage of cell adhesion (b). $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ vs untreated group.

and possesses anti-cancer properties as well (18,19) Comparatively, metabolites luteolin, luteoloside, quercetin, salicylamide, and stearidonic acid were identified in the MCA extract (Table 1). These metabolites reportedly exert an array of therapeutic activities such as anti-tumour, anti-angiogenic (20), and anti-inflammatory activities (21) Stearidonic acid (an omega-3 fatty acid) reduces the proliferation index and induces xenograft prostate cancer cells apoptosis (22). Quantitative phytochemical analysis revealed the highest TFC, TPC, TTC, and antioxidant activity in the MCA extract (Figures 2a and 2b). These major phytoconstituents (flavonoids, phenolic acids, and tannins) found in high amounts in the MCA extract are highly antioxidative.

The MCA and MCM extracts were further investigated *in vitro* for their potential inhibitory abilities against cancer cells. An ideal antineoplastic agent delays tumor formation by selectively inhibiting cancer cell viability and proliferation (23,24). The MCM extract was selectively cytotoxic toward MDA-MB-231 cells (Figure 4), which renders it a model of antineoplastic agent. On the contrary, the MCA extract proved to be ineffective as an ideal antineoplastic agent as it induced significant cytotoxic in the kidney cell line. Reduced cell viability is suggestive of the occurrence of cell death. Two well-studied modes of cell death in multicellular organisms, apoptosis, and necrosis, are particularly attributed to the ability of antineoplastic agents to reduce cell viability. Unlike necrosis, apoptosis is a physiologically favorable mode of cell demise that results in the elimination of old, damaged, or cancerous cells (25) without causing damage to surrounding cells. The decreased viability caused by MCA extract was associated with necrotic cell death in MDA-MB-231 cells. In contrast, exposure to the MCM extract preferably induced apoptotic cell death (Figure 5). In this regard, the MCA extract was discontinued in subsequent anti-metastatic assays because of its nonselective cytotoxic nature and induction of necrotic cell death. Subsequently, the MCM extract was used at sublethal concentrations to investigate its potential as an effective migrastatics agent.

Metastasis is a multifaceted pathological process wherein tumor cells disseminate from primary to secondary anatomical sites (26). Cell migration is a metastatic process that contributes to the dissemination of cells (27). Therefore, a consequent evaluation of the MCM extract showed the suppression of MDA-MB-231 cell migration (Figure 6a) as evidenced by lowered percentage of wound closure (Figure 6b), suggesting the anti-migratory properties of the extract. Matrix metalloproteinases mediate basement membrane and ECM degradation. Inhibiting their expression has great therapeutic value and indicates a drug, compound, or extract's ability to inhibit cell invasiveness (28).

The MCM extract downregulated MMP-2 and MMP-9 expression (Figures 7a and 7b). This implies that MMP regulation is a viable therapeutic target for the methanolic extract of *M. cardiospermoides* in metastatic breast cancer treatment. Inhibitors of the proteolytic activities of MMPs, known as TIMPs, are compact proteins with abundant cysteine residues that regulate matrix remodeling during ECM degradation by forming a stoichiometric complex with MMPs (28). TIMP-1 is an important prognostic marker for tumour migration and invasion because it specifically regulates the activity of MMP-9 (29). The MCM extract upregulated TIMP-1 expression (Figure 7c), thereby conjecturing that the extract also inhibits

the activity of MMP-9 in addition to downregulating its expression. These findings are often drug-, compound-, or extract-specific (30).

This study reports a significant concentration-dependent inhibition of MDA-MD-231 cell adhesion (Figure 7) by the MCM extract, thereby inferring that the extract has anti-adhesion properties in the breast cancer cell line.

Conclusion

The current study revealed abundant phytoconstituents in MCM and MCA and further demonstrated the ability of the MCM extract to inhibit cell migration, invasion, and adhesion, thus rendering the extract a potentially effective anti-metastatic agent.

Authors' contributions

Conceptualization: Vusi Gordon Mbazima and Mante Dolly Kgakishe.
Data curation: Vusi Gordon Mbazima.
Formal analysis: Mante Dolly Kgakishe, Vusi Gordon Mbazima and Marole Maria Maluleka.
Funding acquisition: Leseilane Mampuru.
Investigation: Mante Dolly Kgakishe and Marole Maria Maluleka
Methodology: Vusi Gordon Mbazima and Mante Dolly Kgakishe
Project administration: Vusi Gordon Mbazima and Kgomotso Welheminah Poopedi.
Resources: Leseilane Mampuru.
Software: Mante Dolly Kgakishe.
Supervision: Vusi Gordon Mbazima.
Validation: Kgomotso Welheminah Poopedi and Bernice Monchusi-Khumalo.
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Conflicts of interests

The authors declare no conflict of interest.

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Data availability statement

Upon a reasonable request, data supporting these findings can be made available by the corresponding author.

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