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Antimetastatic activity of *Tarchonanthus camphoratus* leaf extract against MDA-MB-231 triple-negative breast cancer cells and phytochemical profiling by GC-MS

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ABSTRACT

Introduction: Medicinal plants have gained interest as a suitable alternative for the treatment of cancer. This study explored the anticancer properties of *Tarchonanthus camphoratus* extracts, focusing on their potential to inhibit viability, adhesion, and migration of metastatic breast cancer cells.

Methods: The effects of acetone and water extracts of *T. camphoratus* on the viability of metastatic MDA-MB-231 breast cancer cells and normal Vero kidney cells were assessed using the MTT assay. Cell attachment and transwell migration assays were employed to evaluate the extracts' anti-adhesive and antimigratory effects. Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify phytochemical constituents in the extracts.

Results: The acetone extract significantly reduced the viability of MDA-MB-231 cells while sparing normal Vero cells. Additionally, the extract inhibited cell attachment and migration in a dose-dependent manner. GC-MS analysis revealed the presence of phytochemical constituents such as parthenolide, lupeol, and caryophyllene oxide, which have known anticancer activities. The acetone extract significantly inhibited viability, adhesion, and

migration of metastatic breast cancer cells while exhibiting minimal toxicity toward normal Vero cells.

Conclusion: These findings suggest that *T. camphoratus* is a promising source of anticancer phytochemicals and warrants further investigation to elucidate its molecular mechanisms of action.

Key words: *Tarchonanthus camphoratus* acetone extract, phytochemicals, metastasis, breast cancer.

1. INTRODUCTION

Breast cancer is responsible for the majority cancer-related deaths in women around the world. According to the World Health Organisation (WHO), approximately 2.3 million women were diagnosed with breast cancer and 685 000 deaths occurred around the world in 2020. Despite significant breakthroughs in pharmacology and synthetic pharmaceuticals, conventional cancer treatment (including chemotherapy, radiation, surgery, etc.) frequently requires highly toxic agents with several negative side effects [1] . The main toxicity includes the inability to distinguish normal dividing cells from cancer cells, and others include hair loss, anemia, fatigue, bruising, and bleeding [2] . In addition, cancer metastasis has been reported to be the leading cause of cancer-related deaths, especially in developing countries due to the complications associated with metastasis [3] . The metastasis process involves the spread of the primary tumour, intravasation into the bloodstream, and invasion into secondary tumour sites organs[4] . The metastatic potential of breast cancer determines tumour grade and influences prognosis in patients [5] .

Breast cancer utilizes the epithelial-mesenchymal transition (EMT) phenomenon to break through the basal membrane and transverse to distant sites [6] . The EMT is a process that involves the transformation of epithelial cells from an epithelial phenotype to a mesenchymal phenotype that leads to loss of epithelial integrity, increased migration, invasion, and finally metastasis [7, 8]. Adherent molecules such as E-cadherin (CDH1) and N-cadherin (CDH2) are responsible for facilitating the transition from the epithelial state to the mesenchymal state [5] . The matrix metallopeptidase (MMP) family is known to be involved in the degradation of the extracellular matrix (ECM), promoting cancer cell invasion and metastasis [9]. The Notch signalling pathway has been found to be important during breast cancer development and progression [10] . Activation of Notch1 has been reported to promote the expression of MMP-2 and -9 to break down the ECM components [11]. Emerging evidence has indicated hypoxia-induced Notch activation to EMT in breast cancer [12]. Notch enhances the binding of HIF1α to the lysis oxidase (LOX) promoter, stabilizing the secretion of Snail-1 and tissue inhibitor of metalloproteinase 4 (TIMP4) which leads to EMT and metastasis [13, 14] . Metastatic breast cancer, being incurable, therefore, strengthens the need to understand the regulation of EMT of the metastatic cascade for the development of novel targeted therapies.

Due to the inherent qualities of conventional treatments and metastasis complications, natural products, particularly plants, have established themselves as a suitable alternative for cancer treatment [15] . *Tarchonanthus camphoratus* L. (Asteraceae) has received considerable attention due to its widespread biological activities such as the treatment of bronchitis and chest ailments, tired legs and sore feet, stomach ailments, asthma, sinuses, headaches, overanxiety, and heartburn [16] . Additionally, it has been reported to have anti-inflammatory activity in rats induced with lipopolysaccharides [17] . *Tarchonanthus camphoratus* is an evergreen shrub of 9 m high with a thin crown. It is found in the savanna biome and forest grasslands of Angola, Ethiopia, Kenya, Lesotho, Namibia, Somalia, South Africa, Tanzania, Uganda, and Zimbabwe [18] . The cytotoxicity activity and the effects of the plant on apoptosis have been reported in different cell lines, grown in different regions of the world [19–22]. Although it is a rich source of phytochemical constituents, the benefits of the acetone and water extract of *Tarchonanthus camphoratus* leaves on the inhibitory migratory and adhesive properties against the MDA-MB-231 triple-negative breast cancer cell line have not been investigated.

Given the pivotal roles of cell adhesion and migration in breast cancer metastasis, we proposed that *T. camphoratus* leaf extracts might display anti-metastatic activity by disrupting cellular processes responsible for tumour cell adhesion and motility, potentially via mechanisms related to epithelial–mesenchymal transition (EMT) and interactions with the extracellular matrix. To test this hypothesis, the effects of acetone and aqueous extracts of *T. camphoratus* leaves on cell viability, adhesion, and migration were assessed in the metastatic MDA-MB-231 triple-negative breast cancer cell line. Furthermore, the phytochemical components of the most potent antiproliferative extract were tentatively characterised using gas chromatography–mass spectrometry (GC-MS), and GEPIA analysis was employed to examine the relationship between selected genes, breast cancer progression, and patient survival. Collectively, these methods were applied to gain initial insight into the potential biological mechanisms that may underlie the anti-metastatic properties of *T. camphoratus*.

2. METHODS AND MATERIALS

2.1 Plant collection and Authentication

Tarchonanthus camphoratus leaves were harvested and collected from the Northern Cape, in a small town called Warrenton, with permission obtained from the town councillor. The leaves were identified and authenticated, according to local, national and international guidelines

regarding studies involving plants, by Mr E Mathebula, a botanist in the Department of Botany, University of Limpopo, South Africa. The voucher specimen UNIN 1220081 was deposited at the Larry Leach Herbarium (UNIN) at the University of Limpopo.

2.2 *Sample Extraction*

The plant was air-dried on the benchtops in the extraction laboratory followed grounding to a fine powder using a commercial blender. Fine grounded leaves (20 g) were exhaustively extracted using 200 mL of acetone with continuous shaking at 150 rpm for 24 hours at room temperature using a rotary shaker. The resulting acetone extract was filtered using Whatman No. 1 filter paper and air dried under a fan. To mimic the traditional process, 20g of fine ground leaves were extracted using 200 mL of boiled water overnight, as described above. Acetone and water extracts were reconstituted in dimethyl sulfoxide (DMSO) and stored at -20 ° C until further use.

2.3 Cell Culture and Maintenance

In this study, human breast adenocarcinoma cell lines (MDA-MB-231) (ECACC) and African green monkey normal kidney (Vero) were used. Cells were cultured in DMEM (Dulbecco's Modified Essential Medium) (Gibco, NewZealand) growth medium supplemented with 10% fetal bovine serum (HyClone, USA) and 1% penicillin/streptomycin (PSN; Gibco, USA) at 37 ° C in a humidifier atmosphere of 5% CO_2.

2.4 Cell viability cytotoxicity assay

The 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to assess the cell viability of MDA-MB-231 cells during treatment with acetone and water extracts. Cells (1×104 cells/well) were seeded overnight for attachment, followed by treatment. After 24 hours of TCA and TCW treatment (50 µg/mL – 250 µg/mL) and 0.1 % DMSO (vehicle control), 5 mg / mL of MTT solution was added to the wells and further incubated for 2 hours. Following incubation, 100 µL DMSO was added to dissolve formazan crystals, and absorbance was measured at 560 nm using the BioRad 96-well microplate reader. The percentage of cell viability was calculated using the formula:

$$\%\ Cell\ viability = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control}\ X\ 100\ \%$$

2.5 Cell Adhesion Assay

To determine the effect of acetone and water extracts on cell attachment to the surface of cell culture plates in MDA-MB-231 cells, the cell attachment assay was used. The cells were seeded at a density of 1 x 10^5 cells/well and treated (6.25 to 50 µg/mL) for 24 hours. Curcumin was used as a positive control. After each treatment, cells were washed three times with 1 x PBS. Cells were scraped off and reseeded into 24-well plates for 3 hours. Cells were washed twice with 1x PBS. The attached cells were fixed with methanol: acetic acid (3:1) for 5 minutes at 37 ° C and stained with 0.5% crystal violet solution for 15 minutes. Stained cells were photographed under the Olympus light inverted microscope at a magnification of 10x. The stained cells were further quantified by solubilizing the crystal violet-stained cells with DMSO. The percentage of cell adhesion was calculated after measuring the absorbance at 560 nm using the BioRad 96-well microplate reader.

2.6 Transwell Migration Assay

The transwell migration assay was used to assess the effect of the *Tarchonanthus camphoratus* acetone and water extracts on the migration of MDA-MB-231 breast cancer cells. The cells were seeded in 24-well plates and allowed to grow overnight to form monolayers. The migration assay was conducted without the Matrigel coating. Serum-free media (100 µl) were added to the top of each chamber with 8 µm pores, followed by 200 µl of serum-free media containing 2.5 x 10^5/mL cells. In the lower chambers, 750 ul of medium with 10% FBS was added. Cells were treated with 12.5 to 50 µg/mL of the TCA and TCW in the top chamber. The chambers were then incubated for 24 hours at 37 ° C. The migrating cells attached to the lower surface of the filter were fixed with methanol: acetic acid (3:1) and stained with 2% ethanol containing 0.5% crystal violet powder. Cells that invaded or migrated through the membrane were counted under a light microscope (× 40).

2.7 Gas *Chromatography-Mass Spectrometry (GC-MS)*

The chemical composition of the plant extracts was performed using Agilent Gas Chromatography (GC) 8890 coupled to mass spectrometry (MS). The analyses were performed using a Phenomenex column (HP-5MS with 30 m × 0.25 mm × 0.25 µm. 1uL of the plant extract was injected into the GC at 280 ºC using helium as a carrier gas at a constant flow rate of 1.2 mL /min. The oven temperature program used started from 40 ºC and held for 2 min, ramped at 5 ºC/min to a final temperature of 280 ºC and held for 10 min until the analyses is complete. The data collected from the GC-MS was further processed for using Mass hunter for

reprocessing of the chromatograms by means of compounds search using NIST library search database.

2.8 GEPIA survival analysis

To evaluate the effects of genes involved in the metastasis process on the prognosis of BRCA patients over a 250-month period, the GEPIA survival analysis tool (http://gepia2.cancer-pku.cn/#survival) was used. Notch1, MMP-2, MMP-9, HIF1A, and TIMP4 genes were used as input, and overall survival (OS) and disease-free survival (DFS) Kaplan-Meier plots were obtained as outputs. In total, ten plots were constructed.

2.9 ***Statistical analysis***

Each experiment was performed using at least three independent biological replicates, and the results are presented as the mean ± SEM. Statistical analyses were performed using two-way analysis of variance (ANOVA) to assess the effects of extract type and treatment concentration, followed by an appropriate multiple-comparison test. A P-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 *The Effect of Acetone and Water Extracts of T. camphoratus on MDA-MB-231 and Vero Cell Viability*

The effect of the two extracts of T. camphoratus on the cell viability of MDA-MB-231 breast cancer and normal Vero kidney cells was investigated using the MTT assay. Both acetone (TCA) and water (TCW) extracts of T. camphoratus exhibited a dose-dependent reduction in MDA-MB-231 cell viability (Figure 1a). The acetone extract (TCA) demonstrated higher cytotoxicity at concentrations between 100 µg/mL and 250 µg/mL, while the water extract (TCW) showed significant effects at 200 µg/mL and 250 µg/mL. At the highest concentration (250 µg/mL), TCA reduced cell viability to 39% compared to 89% in the control group after 24-hour treatment. In contrast, TCW treatment maintained cell viability above 70%.

In Vero cells, both extracts exhibited minimal cytotoxicity across all tested concentrations, with cell viability remaining above 75% (Figure 1b). For subsequent metastasis-related assays, sub-lethal concentrations (6.25–50 µg/mL) were selected based on observed cell viability > 80%.

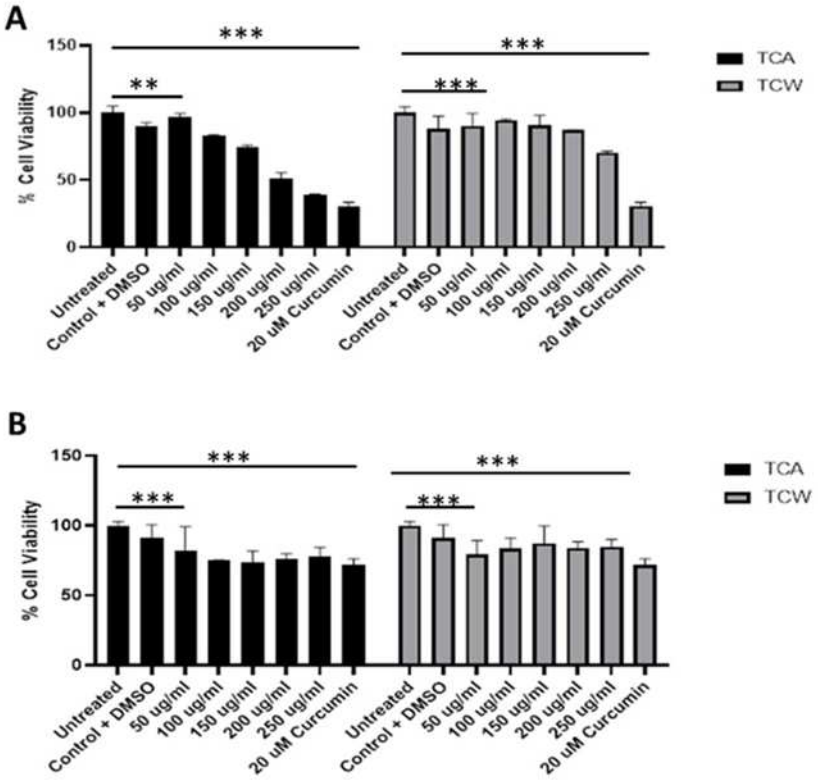

Figure 1: Effects of the acetone and water extract of *T.Camphoratus* on the cell viability of (a) MDA-MB-231 and (b) Vero cells. The cells were treated for 24 hours with and without extracts. Curcumin was used as a positive control. Data are the mean ± SD of three independent experiments. TCA; Acetone extract of *T.Camphoratus*, TCW; Water extract of *T.Camphoratus*.

3.2 *TCA Extract Inhibits the Adhesion of Metastatic MDA-MB-231 Breast Cancer Cells*

The effect of TCA and TCW extracts on the adhesion of MDA-MB-231 cells to the cell culture surface was investigated using the cell adhesion assay. After 24 hours of treatment, TCA significantly reduced cell adhesion. Specifically, 12.5 µg/mL and 25 µg/mL TCA treatments inhibited adhesion by 30.03% and 52.07%, respectively (Figure 2a). These effects were comparable to the positive control, curcumin (52.0%). In comparison, TCW extract reduced cell adhesion by 33.06% and 36% at equivalent concentrations (Figure 2b).

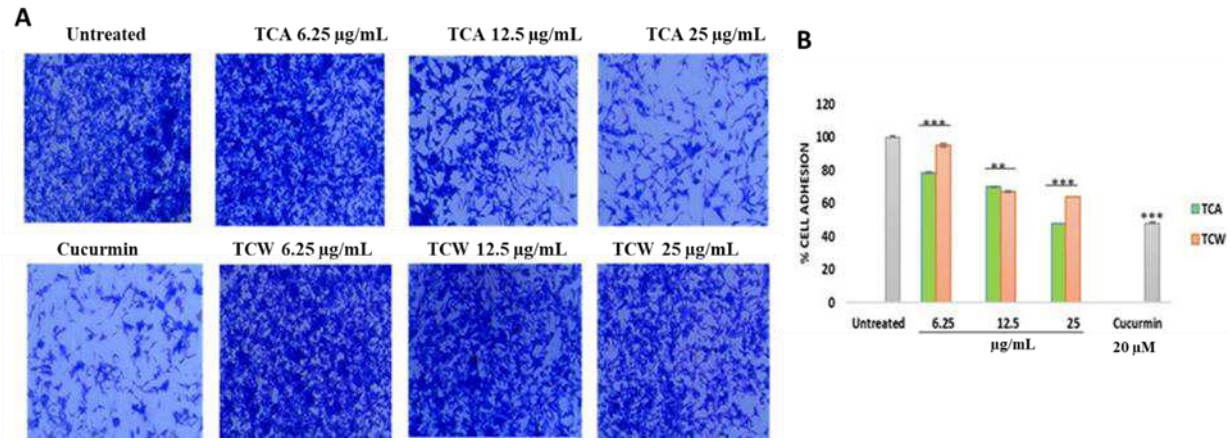

Figure 2: The effect of *T. Camphoratus* acetone **(TCA)** and water **(TCW)** extracts on cell adhesion in MDA-MB-231 breast cancer cells (a). Cells were treated for 24 hours with *T. Camphoratus*, fixed and stained with methanol: acetic acid and crystal violet. Photographs were captured under an inverted light microscope (10×). The quantification of the percentage of adherent cells is shown as a bar graph (b). Data are the mean ± SD of three independent experiments.

3.3 *TCA Extract Inhibits Migration of Metastatic MDA-MB-231 Cells*

A transwell migration assay was employed to evaluate the migratory potential of MDA-MB-231 breast cancer cells after exposure to TCA and TCW extracts. To ensure results were migration-specific and not influenced by invasion, the transwell membrane was not coated with extracellular matrix components. Serum-free medium was added to the top chamber, and no migrated cells were removed from the upper surface of the membrane.

After 24 hours, TCA significantly reduced cell migration by 55% at a concentration of 25 µg/mL, outperforming curcumin, which showed a 36.03% inhibition. In contrast, TCW had a more modest effect, reducing migration by only 33% at the same concentration (Figure 3).

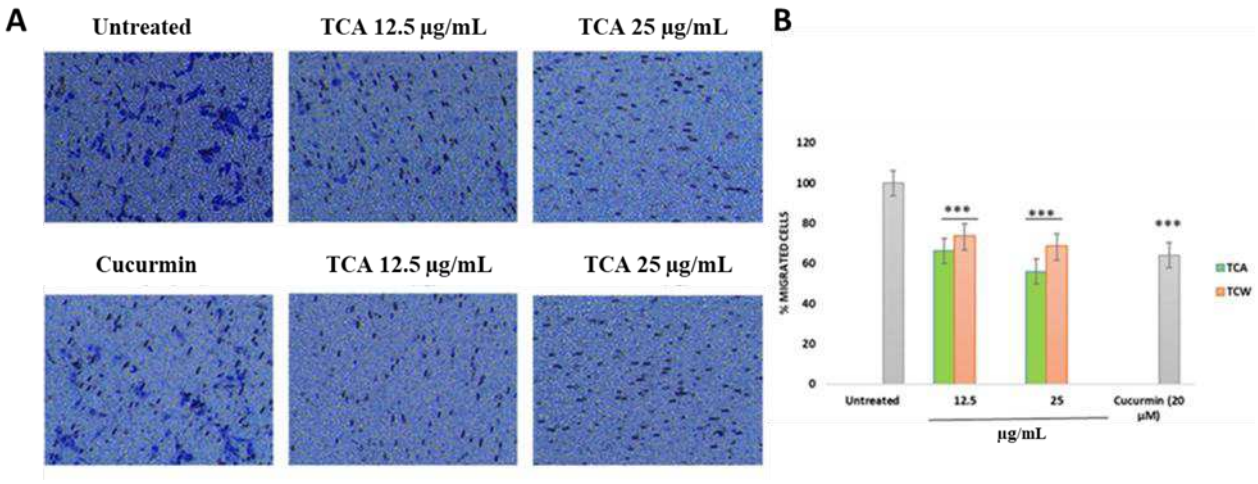

Figure 3: Effects of the acetone extracts **(TCA)** and water extracts **(TCW)** of *T. Camphoratus* on MDA-MB-231 cell migration. Acetone extract of *T. Camphoratus* strongly inhibited the metastatic potential of MDA-MB-231 cells at 25 ug / ml 24 hours after treatment. Photographs were captured under an inverted light microscope (10×).

3.4 *Identification of Phytochemical Constituents in TCA Extract*

GC-MS analysis of the TCA extract tentatively identified more than ten phytochemical constituents with known pharmacological properties. Parthenolide, a sesquiterpene lactone, was detected, along with lupeol, a pentacyclic triterpenoid, and caryophyllene oxide, a phytochemical terpenoid. The chemical structures of these selected compounds are shown in Figure 4, and their retention times are listed in Table 1.

Table 1: Compounds tentatively identified from the *T. Camphoratus* solvent extract using GC-MS.

Retention time (min)	**Compound name**	**Match factor**	**Molecular formular**
16.07	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	81	$C_{11}H1_6O_2$
16.72	Caryophyllene oxide	86	$C_{15}H_{24}O$

18.25	Isospathulenol	76	$C_{15}H_{24}O$
20.72	Naphtho[2,3-b]furan-2(3H)-one, 4a,5,6,7,8,8a-hexahydro-3,8a-dimethyl-5-methylene-	70	$C_{15}H_{18}O_2$
22.05	Cyperanic acid	92	$C_{15}H_{22}O_4$
22.29	Achalensolide	78	$C_{15}H_{18}O_3$
23.14	Reynosin	90	$C_{15}H_{20}O_3$
23.81	Parthenolide	98	$C_{15}H_{20}O_3$
23.92	2H-Cyclohepta[b]furan-2-one, 3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-6-(3-oxobutyl)-, [3aR-(3a.alpha.,7.beta.,8a.alpha.)]-	88	$C_{15}H_{20}O_3$
24.57	Methyl 2-(2-hydroxy-3-(3-hydroxyprop-1-en-2-yl)-6-(isobutyryloxy)-4-methyl-4-vinylcyclohexyl) acrylate	81	$C_{20}H_{30}O_6$
25.16	Ageratriol	81	$C_{15}H_{24}O_3$
25.22	Uvidin C, diacetate	87	$C_{19}H_{30}O_5$
25.31	Octane, 2,7-dimethyl-	80	$C_{10}H_{22}$
25.31	3,5-Dimethyldodecane	76	$C_{14}H_{30}$
25.97	4-(2-Hydroxy-2,5,5,8a-tetramethyldecahydro-1-naphthalenyl)-3-buten-2-one	74	$C_{18}H_{30}O_2$
38.66	Lup-20(29)-en-3-one	89	$C_{30}H_{48}O$
39.28	Epilupeol; 20(29)-Lupen-3alpha-ol (isomer 1)	72	$C_{30}H_{50}O$
39.29	Lupeol	81	$C_{30}H_{50}O$

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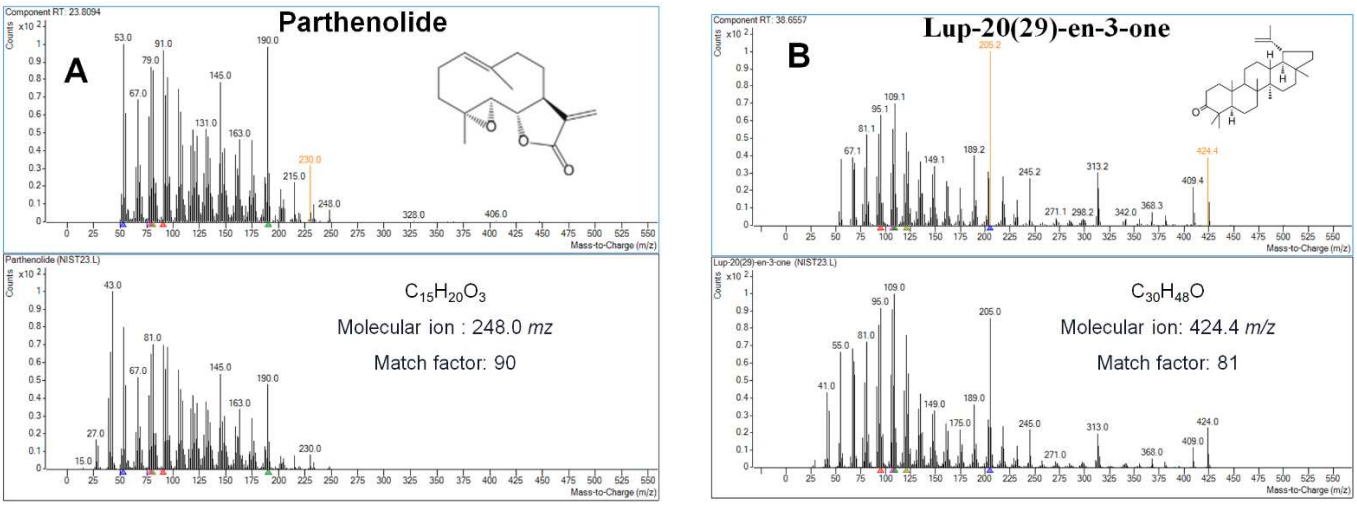

Figure 4: Examples of some mass spectra obtained for the plant extract corresponding to: **A).** Parthenolide, **B).** Lup-20(29)-en-3-one.

3.5 *Survival Analysis of Metastasis-Associated Genes*

The expression of several metastasis-associated genes—Notch1, MMP-2, MMP-9, HIF1A, and TIMP4—was evaluated using the GEPIA survival analysis tool (http://gepia2.cancer-pku.cn/#survival) to determine their relationship with overall survival (OS) and disease-free survival (DFS) in breast cancer patients. High Notch1 expression was associated with longer overall survival, although no significant difference was observed in disease-free survival. Conversely, low MMP-2 expression correlated with both longer OS and DFS, indicating its potential role as a negative prognostic factor. Interestingly, MMP-9 exhibited the opposite trend, with high expression linked to improved overall and disease-free survival outcomes. For HIF1A, high expression was associated with better disease-free survival, while no notable difference in overall survival was detected. Finally, high TIMP4 expression was related to poorer disease-free survival and shorter overall survival, suggesting a detrimental effect on patient prognosis (Figure 5).

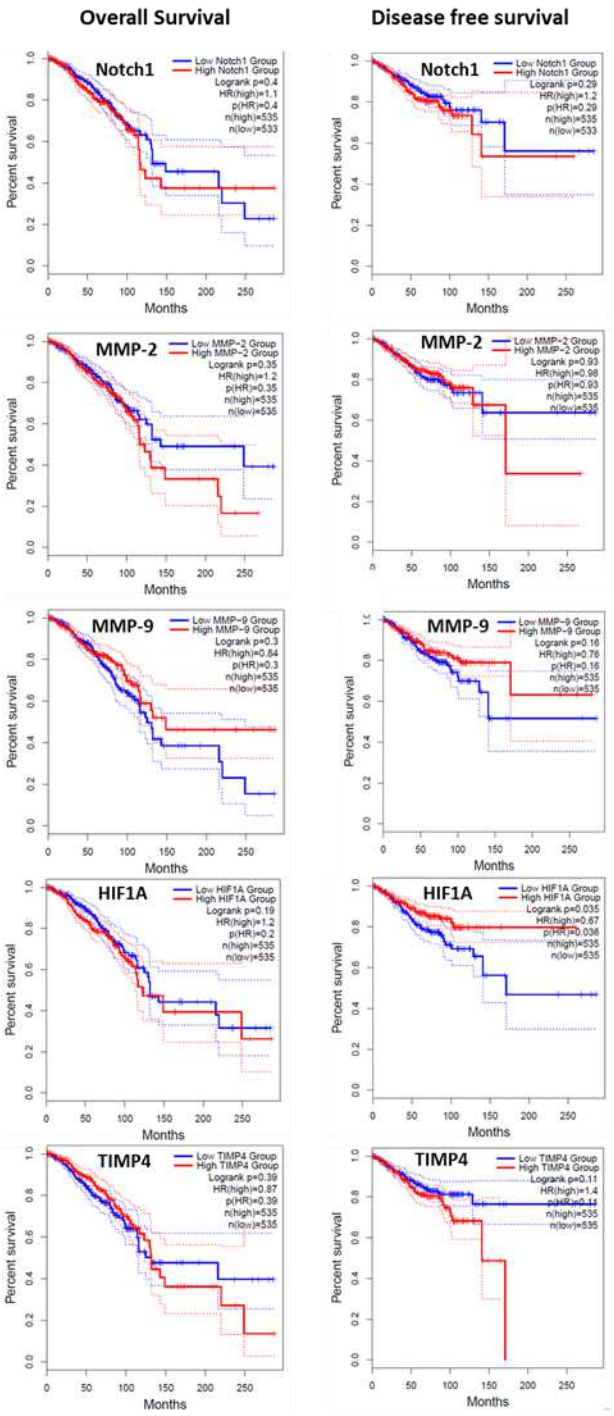

Figure 5: Kaplan-Meier graphs showing overall survival (OS) of the patient and disease-free survival (DFS) of BRCA patients. The plots represent OS and DFS over a period of 250 months. The expression of metastatic genes in tumour tissue and the survival prognostic of the patients were investigated using the GEPIA survival analysis tool (http://gepia2.cancer-pku.cn/#survival). The red lines represent high-expression survival data, while the blue lines represent low-expression survival data.

4. DISCUSSION

Compounds from medicinal plants have shown potency as anticancer agents as they possess cytotoxic effects against cancer cells with minimal side effects. To date, approximately 35,000 plant species have been screened for their anticancer activities, and more than 3,000 plant species were found to reduce cancer cell viability [23, 24] . Medicinal plants contain a wide range of secondary metabolites, including flavonoids, flavones, lignans, coumarins, catechins, anthocyanins, and isocatechins [25]. Phenolic components are effective antibacterial, antioxidant, anti-inflammatory, and anticancer agents [26]. Most phenolic compounds are flavonoids that are glycoside, aglycone, and methylate derivatives [27]. In the present study, the effects of *Tarchonanthus camphoratus* acetone and water leaf extracts were investigated for their antimetastatic effect on metastatic MDA-MB-231 breast cancer cells. To date, there have been no reports on the chemical constituents of the plant grown in the northern parts of South Africa.

The current study provides comprehensive evidence that the *Tarchonanthus camphoratus* acetone extract (TCA) contains potent phytochemical constituents capable of exerting significant anticancer effects against metastatic MDA-MB-231 breast cancer cells. The dose-dependent inhibition of MDA-MB-231 cell proliferation and the significant induction of apoptosis emphasize the extract's potential as an effective anticancer agent, particularly against aggressive triple-negative breast cancer subtypes. The apoptotic induction suggests that mitochondrial pathways and caspase activation are key components of TCA's mechanism, an area warranting further molecular investigation.

A critical aspect of breast cancer progression is metastasis, particularly the migratory capacity of tumor cells. The TCA extract's superior inhibition of MDA-MB-231 cell migration compared to curcumin and the water extract (TCW) highlights its promise as an anti-metastatic agent. Given that metastasis involves complex processes such as epithelial-mesenchymal transition (EMT) and matrix degradation [4, 28] , these results suggest that TCA's phytochemicals may modulate related signaling cascades, thereby impairing cellular motility. Consistent evidence from other studies shows similar migration inhibition by plant extracts, such as the acetone extract of Momordica balsamia in colon cancer cells [29] and Ricinus communis fruit extract in breast cancer cells [30].

GC-MS analysis of the TCA extract revealed several key phytochemicals—including parthenolide, lupeol, and caryophyllene oxide—that lay a strong foundation for understanding

the molecular basis of the observed bioactivities. These compounds have been linked to mechanisms such as apoptosis induction, cell cycle arrest, and migration inhibition, which support the multifaceted anticancer properties of the extract [31–34] .

Complementing these in vitro findings, survival analyses of metastasis-associated genes provided clinically relevant context for their potential relevance to breast cancer progression. The differential expression of genes such as Notch1, MMP-2, MMP-9, HIF1A, and TIMP4 showed variable correlations with overall and disease-free survival in breast cancer patients. High TIMP4 expressions were linked to poor prognosis, underscoring its role in tumor progression and metastasis [35, 36] . Although TIMPs typically inhibit metalloproteinases, TIMP4 overexpression paradoxically promotes stemness and invasiveness in cancer cells [37]. Interestingly, curcumin treatment in MDA-MB-231 cells increased TIMP4 expression [38] (Hassan and Daghestani, 2012), which might explain its comparatively lower inhibition of migration in this model. These results offer a possible biological framework for understanding the anti-migratory effects seen after TCA treatment. Nonetheless, this study did not directly measure TIMP4 expression or activity in response to TCA, so a definitive regulatory link between TCA and TIMP4 cannot be confirmed. Similarly, the survival associations identified in the public breast cancer dataset should be viewed as clinical correlations rather than proof that these genes play a mechanistic role in the anti-metastatic action of TCA. Additional investigations examining gene and protein expression, along with functional validation studies, will be necessary to determine whether TIMP4 or other metastasis-related pathways are involved in the effects of TCA.

Taken together, these results indicate that the anticancer effects of *T. camphoratus* extracts likely stem from the combined actions of several phytochemical constituents that suppress cell proliferation, trigger apoptosis, and limit cancer cell migration. Additional molecular studies and in vivo experiments are needed to confirm these mechanisms and evaluate their therapeutic value in breast cancer.

5. CONCLUSIONS

This study highlights the anticancer and anti-metastatic potential of the acetone extract of *Tarchonanthus camphoratus* (TCA) against the highly aggressive MDA-MB-231 triple-negative breast cancer cell line. Compared with both the water extract and curcumin, TCA more effectively reduced cell viability, triggered apoptosis, and suppressed cell migration. These effects are likely due to the combined action of several phytochemical constituents such

as parthenolide, lupeol, and caryophyllene oxide tentatively identified via GC-MS analysis. Although the findings suggest that TCA may influence pathways associated with metastatic progression, including EMT-related mechanisms, EMT inhibition was not directly assessed in this study. Further mechanistic and in vivo studies are therefore required to validate the underlying mechanisms and determine the therapeutic potential of T. camphoratus in breast cancer.

DECLARATIONS

Abbreviations

BC – Breast Cancer

ECM – Extracellular Matrix

EMT – Epithelial-Mesenchymal Transition

GC-MS – Gas chromatography-mass spectroscopy

HIF1α – Hypoxia-Inducible Factor 1-alpha

MDA-MB-231 - Human triple-negative breast adenocarcinoma cell line

MMP – Matrix Metallopeptidase

Notch1 – Notch homolog 1 (associated with signaling pathway)

TC – *Tarchonanthus camphoratus*

TCA - Tarchonanthus camphoratus Acetone extract

TCW -Tarchonanthus camphoratus Water extract

TIMP4 – Tissue Inhibitor of Metalloproteinases 4

TNBC – Triple-Negative Breast Cancer

Vero - African green monkey kidney epithelial cell line

WHO – World Health Organization

Ethics approval and consent to participate

Tarchonanthus camphoratus was collected from public land in Warrenton, Northern Cape, South Africa, with permission obtained from the town councillor. As commercially available cell lines were used in this study, human research ethics approval was not required.

Consent for publication

Not applicable.

Availability of data and materials

Additional data is available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests

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Authors' contributions

Bernice Monchusi: conceptualization, designed the experiments, carried out the experiment, Formal analysis, wrote and revised the manuscript. **Sithandile Ngxangxa**: Experimented, Formal analysis, wrote and revised the manuscript. Marole Maluleka: Experimented, Formal analysis, wrote and revised the manuscript. **Vusi Mbazima:** Conceptualization, designed the experiments, wrote, and revised the manuscript. **Clement Penny:** Supervision, write and revised manuscript, provided resources.

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