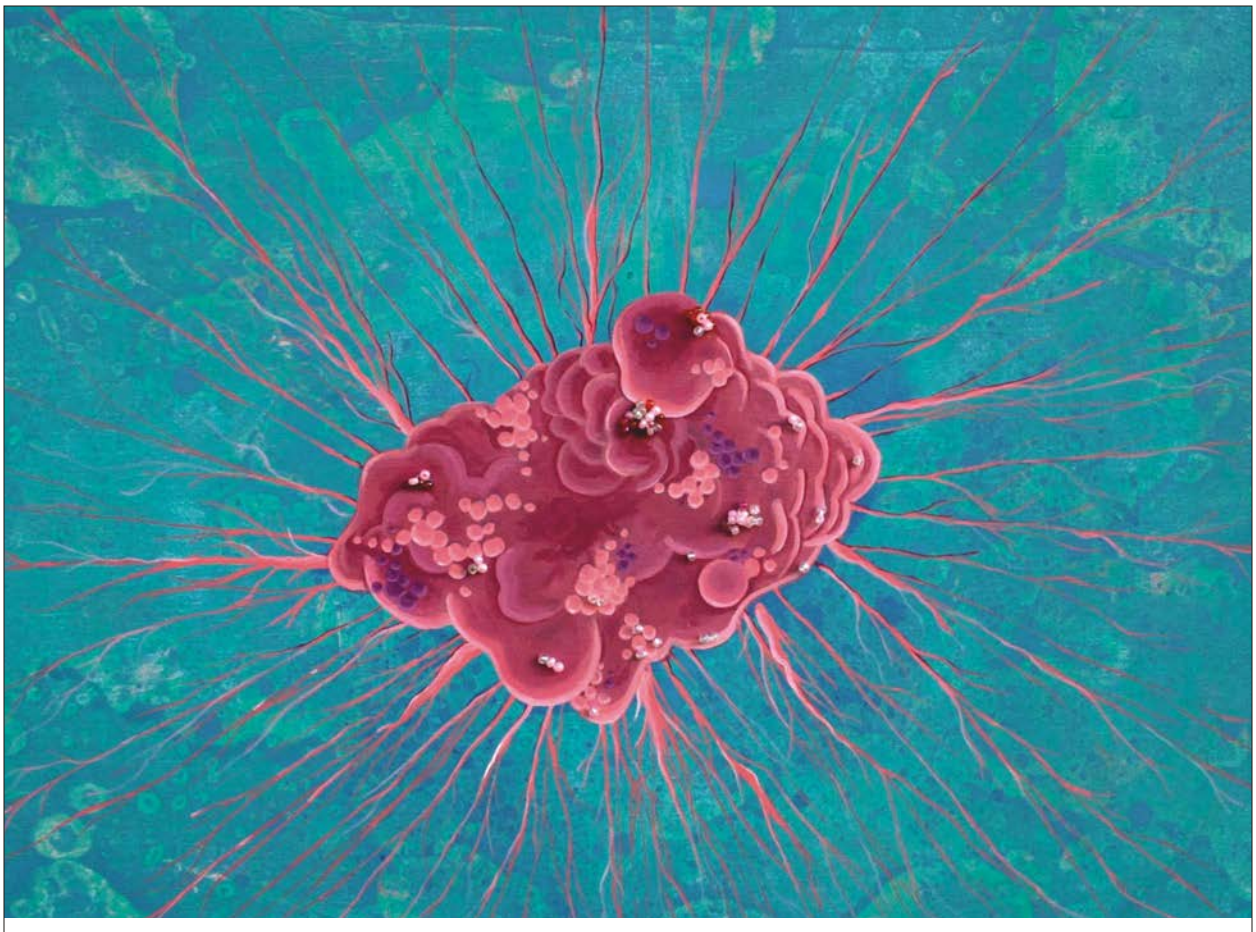

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Anti-Cancer Effect and Mechanisms of Action of *Mikania cordata* Plant Extract on MCF-7 Human Breast Adenocarcinoma Cells

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ABSTRACT

Mikania cordata is a medicinal plant traditionally used as an antibacterial, anti-ulcer, anti-inflammatory, antihelmintic and analgesic agent. The potential of *M. cordata* as an anti-cancer agent has not been explored thus far. This study aims to assess *M. cordata* as a possible anti-cancer agent, specifically against MCF-7 breast cancer cells. Ethanol extract-vacuum liquid chromatography fractions inhibited proliferation of the MCF-7 cells via different mechanisms. Fraction 6 induces non-specific cytotoxicity via apoptosis and oxidative pathways demonstrated by the TUNEL and DPPH assays. Fractions 7, 10 and 11 exhibit selective cytotoxicity against MCF-7 breast cancer cells but does so through mechanisms other than apoptosis and oxidation. The results suggest that novel mechanisms of action may be at work, imparting cancer-specific cytotoxicity properties of these fractions. Phytochemical screening suggests terpenes and saponins as the possible anti-cancer agent in *M. cordata*.

Key words: Mikania cordata, breast cancer, MCF-7, apoptosis, oxidation

INTRODUCTION

Breast cancer is the second most commonly diagnosed cancer worldwide, comprising 11.9% of all new cancers diagnosed. It is currently the leading cause of cancer-related death among women, accounting for 522,000 deaths in 2012 alone (Ferlay *et al*., 2013). Despite the advances in diagnosis and treatment strategies, the trend established over the past decade continues to show increasing incidence and mortality rates. A major reason for treatment failure has been attributed to the development of resistance to the chemotherapeutic drugs used for therapy (Moreno-Aspitia and Perez, 2009; Marquette and Nabell, 2012). This highlights the need for discovery of new and effective drugs to treat breast cancer.

There has been growing interest in the anticancer activities of medicinal plant extracts, due to the presence of secondary metabolites that produce definite pharmacological actions on the human body with lesser side effects (Briskin, 2000). This approach has given rise to the identification of vinca alkaloids, vinblastine and podophyllotoxins as anticancer compounds (Cragg and Newman, 2005).

Mikania cordata (Burm. f.) B.L. Robinson is a creeping woody perennial of the family Ateraceae, widely distributed across Southeast Asia and Eastern Africa. It is more popularly known as heartleaf hempvine, climbing hempvine or mile-a-minute plant and has been described in the

compendium of the world's worst weeds (Mercado, 1994; Nayeem *et al*., 2011). The plant has traditionally been used as herbal medicine to treat pain, inflammations and other infectious disease. Various studies have reported the antibacterial activity of its ethanol leaf extract, analegsic activity of its sesquiterpene dilactone, antiulcer activity of its alkaloidal ethanolic fraction, as well as its use in phytoremediation (Paul *et al*., 2000; Ahmed *et al*., 2001; Mahmud *et al*., 2008; Ali *et al*., 2011). Its sesquiterpene lactone scandenolide has also been found to inhibit inflammatory mediators such as leukotrienes and platelet activating factor synthesis in leukocytes (Ysrael and Croft, 1990). It also causes enhancement of drug-detoxifying enzymes in the liver and increased rate of hepatic proteins synthesis (Mandal *et al*., 1992; Bishayee and Chatterjee, 1995). Although, anti-tumor activity against Hela and K562 cell lines has been demonstrated for *Mikania micrantha* plant extract of same genus, no anticancer studies have been done on *Mikania cordata* species despite the identification of significant chemical constituents in a Gas Chromatographic Mass Spectrometry analysis (Dou *et al.,* 2014; Patar and Yahaya, 2012). Thus, research testing the anticancer activity of *Mikania cordata* is warranted.

This study hypothesized that *M. cordata* exerts anti-cancer effects on human breast cancer cells while reducing toxicity to normal cells and that its anti-cancer activity is mediated through the induction of apoptosis and oxidation. Based on this hypothesis, the study aimed to explore the potential of Vacuum-Liquid Chromatography (VLC)-fractionated ethanolic extracts of leaves of *M. cordata* as anti-cancer agents against MCF-7 breast cancer cell line, evaluate its cytotoxic activity to normal cells using J774A.1 mouse macrophage cell line and brine shrimp lethality assay, study its possible mechanisms of action through apoptosis and oxidation and identify its active components through phytochemical screening and Thin Liquid Chromatography (TLC).

MATERIALS AND METHODS

Solvent extraction of *M. cordata* plant extract: Harvested fresh leaves (396 g) were cleaned, cut into small pieces, air-dried for 7 days and homogenized by a blender (Osterizer). The resulting powder was soaked in distilled methanol for 24 h and filtered using 20-25 μm pore sized Whatman filter paper. The filtrate was concentrated in a rotary evaporator at 45°C (IKA digital evaporator) and the brown condensate was left to air-dry. The dried brown condensate was dissolved in distilled water (AMT) and sonicated (Skymen) at Room Temperature (RT) before performing solvent extraction. Fractions were obtained in increasing polarity using hexane, ethyl acetate and distilled water, accordingly. To obtain hexane fraction, extract was vigorously mixed in 2 parts hexane: 1 part distilled water solvent in a separating funnel. The hexane layer was collected and concentrated in rotary evaporator (45°C, IKA digital evaporator). The hexane distillate obtained from this process was used to exhaust the aqueous layer until it became colorless, after which the clear, organic upper layer was collected as 'hexane fraction'. To obtain ethyl acetate fraction, the aqueous layer from the hexane partitioning was vigorously mixed with 2 parts equivalent ethyl acetate in a separating funnel. The ethyl acetate layer was similarly collected and concentrated in rotary evaporator (45°C, IKA digital evaporator) and used to exhaust the aqueous layer, after which brown layer was collected as 'ethyl acetate fraction'. The final aqueous layer was concentrated by lyophilization (Labconco) to obtain the 'aqueous fraction'. The hexane, ethyl acetate and aqueous fractions were then tested for cytotoxic activity against MCF-7 breast cancer cells.

Vacuum liquid chromatography of *M. cordata* ethyl acetate fraction: The ethyl acetate fraction was further subjected to Vacuum Liquid Chromatography (VLC) to obtain 13 fractions. The

stationary phase was prepared by evenly suspending the ethyl acetate fraction (6.00 g) in silica powder (200 g) and minimal ethyl acetate. This was added to the top of a pre-vacuum pressed silica in a column (5 cm diameter, 20 cm height). The eluent for each mobile phase (250 mL) was concentrated by rotary evaporator (45°C, IKA digital evaporator) and air-dried to solid form. Each plant extract fraction was prepared as 4 mg mL^{-1} in dimethyl sulfoxide (DMSO; Amresco), filter-sterilized through 0.2 μm Whatman filter and tested for cytotoxic activity against MCF-7 breast cancer cells.

Cell lines: *Homo sapiens* breast adenocarcinoma cell line MCF-7 and *Mus musculus* normal macrophage cell line J774A.1 were purchased from American Type Culture Collection (ATCC). MCF-7 was cultured in Minimum Essential Media (MEM; Gibco, Life Technologies), supplemented with 10% Fetal Bovine Serum (FBS; Gibco Life Technologies), 0.01 mg mL^{-1} human recombinant insulin (Gibco Life Technologies) and 1% penicillin-streptomycin (Gibco Life Technologies) in T-25 flasks (Corning). J774A.1 was cultured in Dulbecco's Minimum Essential Media, high glucose with L-glutamine (DMEM; Gibco, Life Technologies), supplemented with 1% v/v 1 mM sodium pyruvate, 1% v/v 48% D(+)-glucose solution, 2% v/v 7.5% sodium bicarbonate, 10 FBS and 1% penicillin-streptomycin (Gibco, Life Technologies) in T-25 flasks. All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2.

Resazurin cell proliferation assay: To rapidly determine which solvent-extracted fraction of *M. cordata* exerted cytotoxic activity against breast cancer cells, the resazurin cell proliferation assay was performed. MCF-7 cells (1.9×10^4 cells/well) were seeded into 96 well plates using fresh culture medium for 24 h at 37°C, 5% CO_2 incubating condition. Seeded cells were confirmed viable by microscopic examination, then treated with filter-sterilized hexane, ethyl acetate or aqueous fraction. Positive control was 8 M hydrogen peroxide while negative control was DMSO diluent. After incubation for 72 h, 20 μL of resazurin dye (Life Technologies) was added, followed by 24 h incubation at RT. Absorbance was measured at 570 nm using a microplate reader (LabeximLedetect 96). Relative cytotoxicity was computed as the ratio of the absorbance of sample to the absorbance of the negative control or untreated sample:

$$\text{Cyt} = \frac{A_{spl}}{A_{NC}} \times 100 \quad (1)$$

MTT cell proliferation assay: The effect of VLC-fractionated ethyl acetate extract of *M. cordata* on MCF-7 and J774A.1 cell viability was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay. The cells were seeded into 96 well plates at 1.14×10^4 cells/well for MCF-7 and 7.6×10^4 cells/well for J774A.1, using fresh culture medium for 24 h at 37°C, 5% CO_2 incubating condition. Seeded cells were confirmed viable by microscopic examination, then treated with increasing concentrations of filter-sterilized plant extract (50, 25, 12.5, 6.25 μg mL^{-1}) in triplicate. Positive control was doxorubicin (DBL), while negative control was DMSO (Amresco) diluent. After incubation for 72 h, 20 μL of 5 mg mL^{-1} MTT (Amresco) was added, followed by 4 h incubation at 37°C, 5% CO_2 until microscopic examination of purple precipitate. Then, 150 μL of dimethyl sulfoxide was added, after which plate was incubated in the dark at RT for 10 min. The formazan product of MTT was measured as absorbance at 570 nm using

a microplate reader (Labexim Ledetect 96). The IC_{50} was determined as the concentration of treatment showing 50% cell growth inhibition in comparison to the control cell growth.

Brine shrimp lethality assay: Cytotoxicity of plant extract was tested on organismal level using the Brine Shrimp Lethality Assay (BSLA). *Artemia salina,* brine shrimp eggs were grown in a beaker with artificial sea water, prepared by dissolving 38 g sea salt in 1 L distilled water. The beaker was aerated under lamp for 48 h to hatch and mature eggs as nauplii (larva). Ten brine shrimps were introduced into each well of 24 well plates. Filter-sterilized plant extract was added to duplicate wells to make concentrations of 1000, 100 and 10 μg mL^{-1}, using artificial seawater to adjust volume to 500 μL per well. Positive control was 10% ethanol, while DMSO was the negative control. The plates were left uncovered under lamp for 24 h, after which the number of surviving shrimps was counted. Percent death was calculated by dividing the number of dead nauplii by the total number and then multiplied by 100. Using probit analysis, the Lethality Concentration (LC_{50}) was assessed at 95% confidence interval. Following Meyer *et al*. (1982) a plant extract with LC_{50} value less than 1000 μg mL^{-1} was considered toxic, while LC_{50} value greater than 1000 μg mL^{-1} was non-toxic (Meyer *et al*., 1982).

TUNEL assay: To test if mechanism for anticancer activity involved apoptosis, terminal deoxynucleotidyl transferase-dUTP nick end labeling, TUNEL assay was performed. MCF-7 (1.14×10^4 cells/well) were seeded into 96 well plates (Corning Costar) using fresh culture medium for 24 h at 37°C, 5% CO_2 incubating condition. After 24 h, seeded cells were confirmed viable by microscopic examination, then treated with increasing concentrations of filter-sterilized plant extract (50, 25, 12.5, 6.25 μg mL^{-1}) in triplicate. Positive control was doxorubicin, while negative control was DMSO. After 72 h, the Click-iT® TUNEL Alexa Fluor® imaging assay (Life Technologies) was then conducted according to manufacturer's instructions. Finally, the plate was imaged under Axio Observer inverted microscope (Carl Zeiss), using excitation wavelength of 350 nm and emission wavelength of 460 nm to view Hoechst-33342 and excitation wavelength of 495 nm and emission wavelength of 519 nm to view Alexa-Fluor® 488.

DPPH oxidation assay: To test if mechanism for anticancer activity involved oxidation, a DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used. DPPH solution (0.12 mg mL^{-1}) (Sigma-Aldrich) was prepared in absolute ethanol in a tube covered with aluminum foil, to prevent reacting with light (Blois, 1958). In each well of a 96-well plate, 90 μL of DPPH solution was dispensed, followed by addition of 10 μL of filter sterilized plant extract to final concentration of 400 μg mL^{-1}, in triplicate. Positive control was gallic acid (Sigma), while negative control was distilled water. This was performed in the dark to prevent light-induced discoloration. The plate was covered with parafilm and aluminum foil, mixed laterally for 30 sec and incubated at 37°C for 30 min, after which absorbance was measured at 570 nm using a microplate reader (Labexim Ledetect 96). Free radical scavenging activity (%) was computed by Eq. 2, where, A_{H2O} is the absorbance of distilled water, A_{spl} is absorbance of sample and A_{PC} is absorbance of positive control of ascorbic acid:

$$FRSA(\%) = \frac{A_{H2O} - A_{spl}}{A_{H2O} - A_{PC}} \times 100 \quad (2)$$

Phytochemical screening: The phytochemical screening for tannins, saponins, terpenoids, flavonoids, cardiac glycosides, phenolic compounds, steroids, alkaloids and triterpenes used

procedures with slight modifications from Harborne (1984), Edeoga *et al*. (2005) and Onwukaeme *et al*. (2007). Each plant extract fraction was prepared in DMSO (100 μg uL^{-1}) and used as 'plant sample' for qualitative testing of phytochemical constituents.

Thin liquid chromatography: Each plant extract fraction (4 mg mL^{-1}) was dissolved in a sufficient amount of ethyl acetate and was plated to a 6×4 cm silica gel thin layer chromatography plate. This was placed in a saturated gas chamber with shallow layer of 50% ethyl acetate and 50% hexane solvent. The solvent was allowed to rise till the 6 cm mark on the plate then removed for reading. The Retention factor values were obtained through the Iodine staining and visualization under ultraviolet light (254 nm).

Statistical analyses: Results were analyzed in SPSS (IBM SPSS Version 20) using one-way analysis of variance (ANOVA) and Duncan's multiple range test as post hoc analysis. Differences were considered statistically significant at the level of p-value<0.05.

RESULTS

***Mikania cordata* fractions 6, 7, 10 and 11 inhibited proliferation of MCF-7 breast cancer cells:** To rapidly determine which solvent extracted fraction of *M. cordata* exerted anticancer activity against breast cancer cells, resazurin cell proliferation assay was performed. Figure 1 shows the relative percentage cytotoxicity of *M. cordata* hexane, ethyl acetate and aqueous fractions on MCF-7 cell line. The fractions were compared with hydrogen peroxide (H_2O_2) which is known to cause oxidative stress-mediated cytotoxicity in MCF-7 cells (Alarifi, 2011). The ethyl acetate fraction significantly inhibited the proliferation of MCF-7 cells. Furthermore, as with

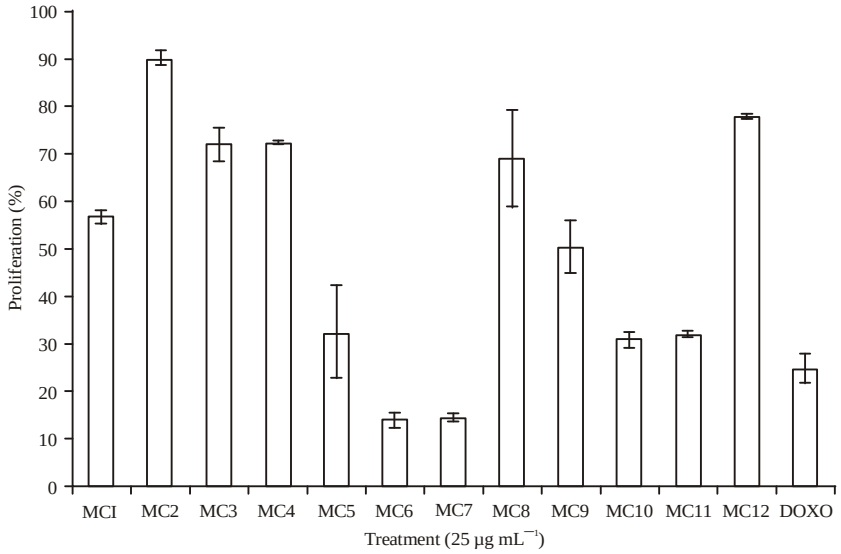

Fig. 1: Effect of VLC-fractionated ethyl acetate *M. cordata* fractions on cell proliferation. Cells were cultured in 96-well plates and treated with 4 mg mL^{-1} VLC fraction for 72 h. Cell viability was measured by MTT assay. Data represent the Mean±SD of three replicates. Statistical differences were analyzed with one-way ANOVA and Tukey's range test

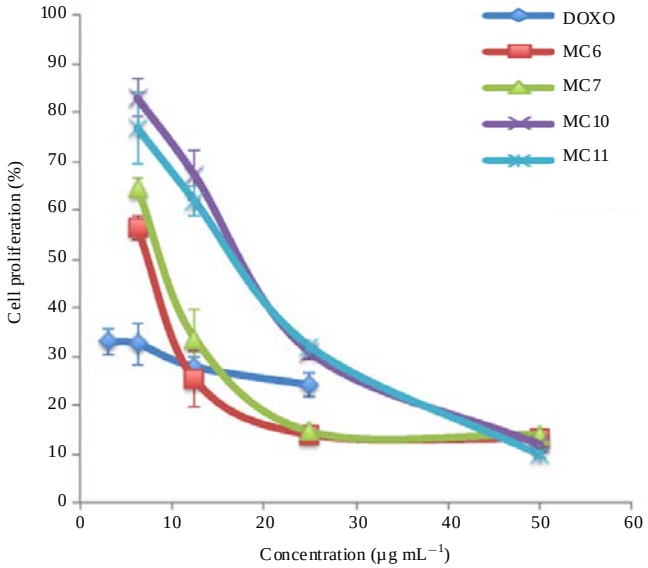

Fig. 2: Anti-proliferative effect of *M. cordata* on human breast cancer cells. Dose response curve of *M. cordata* treatment in MCF-7 cells. Cells were cultured in 96-well plates and treated with indicated concentrations of *M. cordata* VLC fractions for 72 h. Cell viability was measured by MTT assay. Data represent the Mean±SD of three replicates. Statistical differences were analyzed with one-way ANOVA and Tukey's range test

untreated cells, treatment with 1% DMSO diluent had no significant cytotoxic effect on cells; therefore, the cytotoxic effect on MCF-7 was mediated by the plant extract treatment itself.

The ethyl acetate fraction of *M. cordata* was further partitioned by vacuum liquid chromatography. To determine the effect of the *M. cordata* VLC fractions on the proliferation of breast cancer cells, MTT cell proliferation assay was performed. Figure 1 shows the percent cell proliferation of MCF-7 breast cancer cells after treatment with different fractions. The fractions were compared with doxorubicin (DBL), a widely used chemotherapeutic agent for breast cancer (Wang *et al*., 2004). *M. cordata* VLC fractions 6, 7, 10 and 11 significantly inhibited cell proliferation, at levels statistically similar to positive control doxorubicin. Their cytotoxicity curves on Fig. 2 show that inhibition was dosage-dependent. Although, cells treated with *M. cordata* generally had higher level of proliferation compared to positive control doxorubicin, % proliferation was lower in cells treated with 25 µg mL^{-1} of fractions 6 and 7. Results suggest that *M. cordata* fractions 6 and 7 become more potent than doxorubicin but only at higher concentrations. Figure 3 compares the IC_{50} values of all fractions on MCF-7 cells which is shown in Table 1. Half maximal Inhibitory Concentration (IC_{50}) values are commonly used to evaluate the potency of a compound, in which the lower the IC_{50} value, the compound is more potent.

***Mikania cordata* fractions 10 and 11 showed less cytotoxicity to normal J774A.1 murine macrophages:** To assess the effect of *M. cordata* fractions on normal cells, MTT cell proliferation assay was performed on a normal cell type, the J774A.1 murine macrophage cells (Blasi *et al*., 1987). The cytotoxicity curves of *M. cordata* fractions 6, 7, 10 and 11 in normal murine macrophages in comparison with breast cancer cells. *M. cordata* fractions 6 and 7 inhibited proliferation of both normal and cancer cells, indicative of non-specific cytotoxicity. *M. cordata*

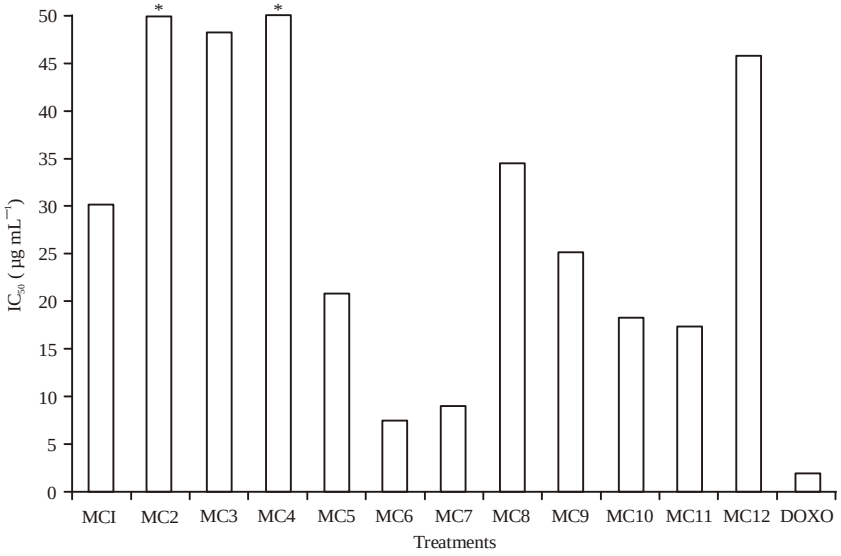

Fig. 3: Half maximal inhibitory concentration (IC_{50}) values on MCF-7 for each VLC fraction. The ceiling value of 50 µg mL^{-1} was estimated, when IC_{50} could not be interpolated by linear regression in MC fractions 2 and 4

Table 1: Half maximal inhibitory concentration values on MCF7 and J774A.1 cells for each VLC fraction

	IC_{50} (µg mL^{-1})	
Treatments	MCF7 cells	J774A.1 cells
MC1	30.297	24.510
MC2	50.00*	40.640
MC3	48.188	23.034
MC4	50.00*	36.354
MC5	20.965	10.899
MC6	7.506	3.631
MC7	9.185	4.890
MC8	34.585	29.240
MC9	25.210	38.942
MC10	18.377	24.124
MC11	17.449	26.540
DOXO	2.041	1.997

*Ceiling value of 50 µg mL^{-1} was estimated, when IC_{50} could not be interpolated by linear regression in MC fractions 2 and 4

fractions 10 and 11, however, inhibited proliferation of cancer cells more than normal cells. Accordingly, as shown in Table 1, IC_{50} was higher for normal cells, compared to the cancer cells.

***Mikania cordata* fractions 10 and 11 showed less toxicity to brine shrimps:** To screen for toxicity to zoologic systems, *M. cordata* fractions were tested on hatched brine shrimp nauplii. The VLC fractions all showed brine shrimp larvicidal activity. The Lethality Concentrations (LC_{50}) are shown in Table 2. Since all the LC_{50} values were less than 1000 µg mL^{-1}, the *M. cordata* fractions were considered toxic by standard Meyer *et al*. (1982). The brine shrimp lethality was dosage-dependent, with maximum mortality (100%) observed at 1000 µg mL^{-1} concentration. However, *M. cordata* fractions 10 and 11 are relatively less cytotoxic to brine shrimp, than fractions 6 and 7.

***Mikania cordata* fraction 6 induced apoptosis in MCF-7 cells:** To determine if anti-cancer activity is mediated by apoptosis, TUNEL assay was performed. Figure 4 shows the apoptotic activity of *M. cordata* fraction 6 on MCF-7 cells, similar to that of positive control, doxorubicin. In

Table 2: Cytotoxic effect of *M. cordata* VLC fractions on brine shrimp

	Percent deaths at (24 h)			
	1000	100	10	LC_{50}
Treatments	($\mu g\ mL^{-1}$)			
DMSO	100	45	10	83.621
MC 1	100	80	40	17.298
MC 3	100	70	45	17.205
MC 4	100	70	30	29.162
MC 5	100	80	35	20.454
MC 6	100	70	50	13.719
MC 7	100	85	45	13.257
MC 8	100	60	35	30.818
MC 9	100	60	30	36.044
MC 10	100	65	30	32.388
MC 11	100	75	35	22.539
MC 12	100	50	20	58.828

Table 3: Phytochemical screening of solvent extracted *M. cordata*

Solvent fraction	Tannins	Saponins	Terpenoids	Cardiac glycosides	Phenolics	Steroids	Alkaloids	Flavonoids
Hexane	-	-	-	-	-	-	-	-
Ethyl acetate	-	+	+	-	-	-	-	-
Aqueous	+	+	+	-	-	-	-	+

Table 4: Phytochemical screening of vacuum liquid chromatography extracted- ethyl acetate fractions of *M. cordata*

VLC fraction	Tannins	Saponins	Terpenoids	Cardiac glycosides	Phenolics	Steroids	Alkaloids	Flavonoids
1	-	+	+	-	-	-	-	-
2	-	+	+	-	-	-	-	-
3	-	+	+	-	-	-	-	-
4	-	+	+	-	-	-	-	-
5	-	+	+	-	-	-	-	-
6	-	+	+	-	-	-	-	-
7	-	+	+	-	-	-	-	-
8	-	+	+	-	-	-	-	-
9	-	+	+	-	-	-	-	-
10	-	+	+	-	-	-	-	-
11	-	+	+	-	-	-	-	-
12	-	-	+	-	-	-	-	-
13	-	-	+	-	-	-	-	-

contrast, more similar to the negative control DMSO, *M. cordata* fractions 7, 10 and 11 did not exert any apoptotic activity on the cells. The anti-cancer activity of *M. cordata* fraction 6 is therefore, mediated by apoptosis.

***Mikania cordata* fraction 6 induced oxidation in MCF-7 cells:** To determine if anti-cancer activity is mediated by oxidation, DPPH assay was performed. Figure 5 shows the Free Radical Scavenging Activity (FRSA) values for each fraction. Fractions were compared to gallic acid which has established antioxidant properties (Bhadoriya *et al*., 2012). No oxidative activity was detected in *M. cordata* fractions 7, 10 and 11; in fact, these fractions enhanced free radical scavenging activity at a level similar to positive control gallic acid. Anti-cancer activity in fractions 7, 10 and 11 is therefore, not mediated by oxidation. In contrast, oxidative activity was detected in *M. cordata* fraction 6 which indicates that its anti-cancer activity may also be mediated by oxidation.

Terpenes and saponins are candidates responsible for anti-cancer activity: Phytochemical screening was performed to identify possible active components responsible for the anti-cancer activity. Table 3 and 4 show the presence of saponins and terpenoids in the active *M. cordata*

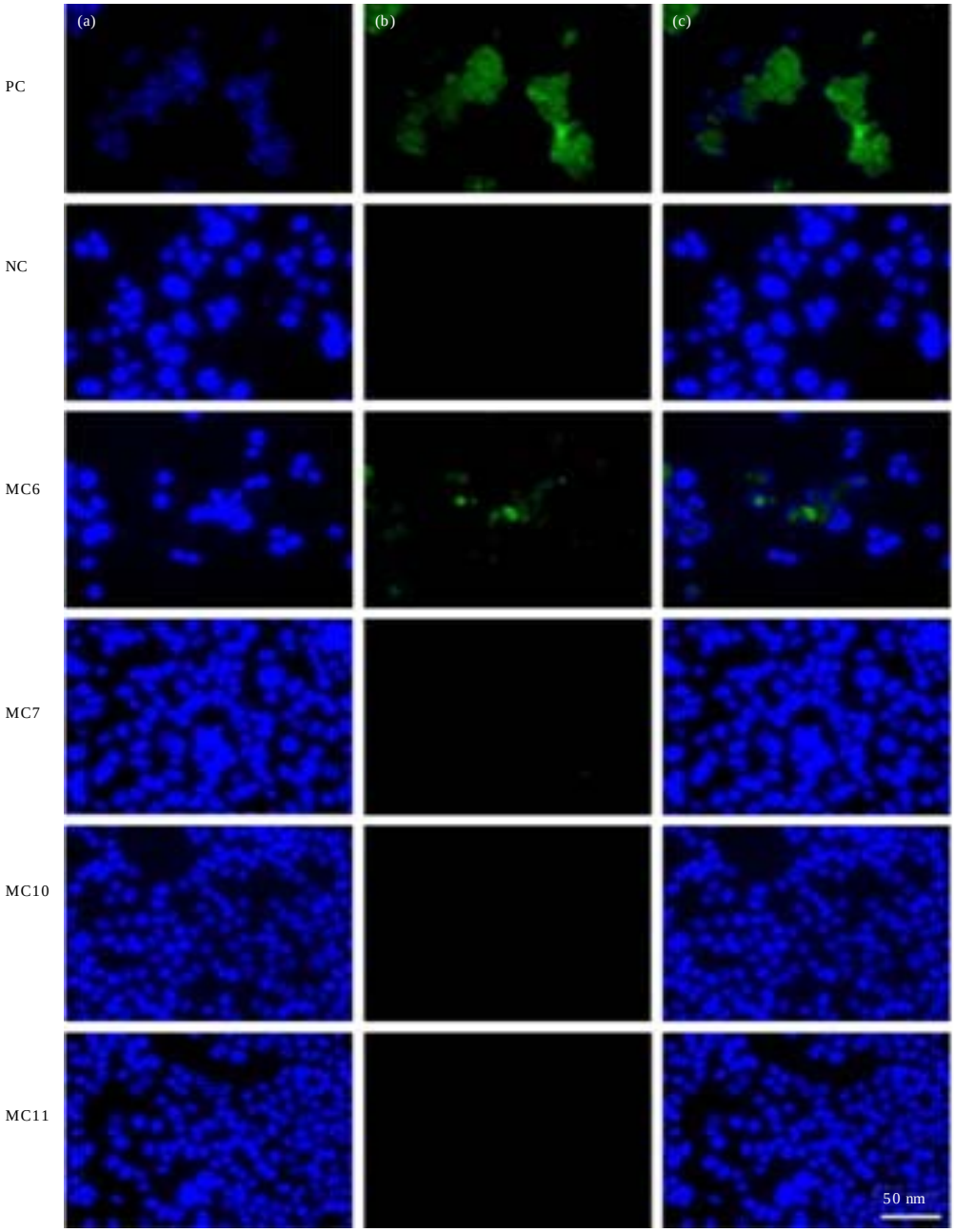

Fig. 4(a-c): Micrographs of apoptotic MCF-7 cells from TUNEL assay. Cells were cultured in 96-well plates and treated with *M. cordata* VLC fractions for 72 h. Cells were subjected to Click-iT® TUNEL Alexa Fluor® imaging assay (Life Technologies). Green fluorescence shows apoptotic cells. PC: Positive control doxorubicin, NC: Negative control DMSO, (a) HOECHST-33342, (b) Alexa-Fluor 488 and (c) Overlay

ethyl acetate fraction and more specifically in the VLC extracted fractions 6, 7, 10 and 11. Such components, however, were similarly present in non-active fractions.

The thin layer chromatography was able to further separate components of each fraction according to polarity as shown in Table 5. The differences in constituent chemical compounds is

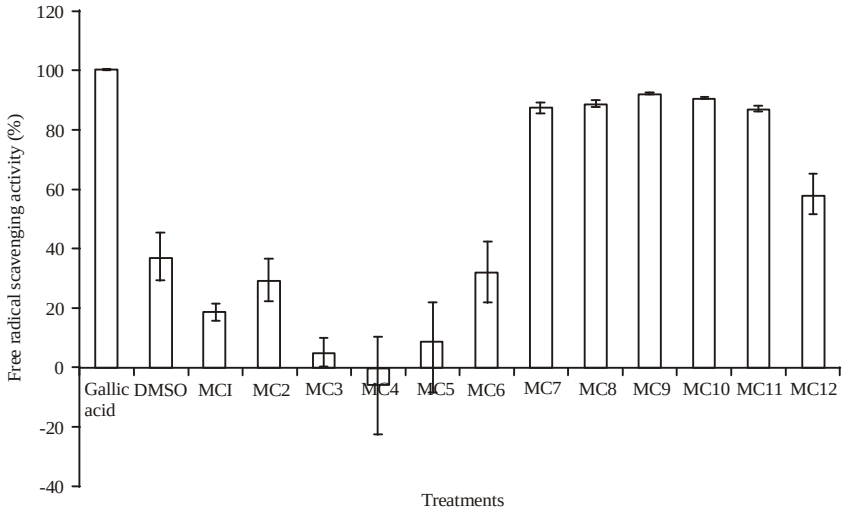

Fig. 5: Free radical scavenging activity of VLC fractions. Fractions were tested for antioxidant capacity using the free radical DPPH

Table 5: Retention factor values from thin layer chromatography of *M. cordata* fractions on silicon gel matrix and 50:50 hexane ethyl acetate eluent

	Rf*										
VLC fraction	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
1	5.2			0.87							
2											
3	5.2			0.87							
4	5.2			0.87							
5	5.2			0.87							
6		0.97	0.88		0.83	0.75					
7		0.97	0.88		0.83	0.75					
8		0.97	0.88				0.5			0.1	
9						0.75	0.5		0.13		
10							0.5		0.13		
11								0.17			0.08
12											0.08
13											

*Rf is defined as the distance traveled by the compound divided by the distance traveled by the solvent

suggested to be responsible for differences in the anti-cancer activity of *M. cordata* VLC fractions, differences in mechanism of action and cytotoxicity to normal cells and brine shrimps of *M. cordata* fractions that are active.

DISCUSSION

Mikania cordata is an important medicinal plant that has been used in different traditional systems of medication for treating human disease. Its extract has accordingly been found to exert antibacterial, anti-ulcer, anti-inflammatory, anthelminthic and analgesic effects (Paul *et al*., 2000; Ahmed *et al*., 2001; Mahmud *et al*., 2008; Ali *et al*., 2011). However, no anti-cancer studies have been done on *M. cordata* species, despite identification of significant chemical constituents in a Gas Chromatographic Mass Spectrometry analysis (Dou *et al*., 2014; Patar and Yahaya, 2012). The present study sought to explore the potential of *M. cordata* as anti-cancer agents against MCF-7 breast cancer cell line.

The results show that some of the Vacuum-Liquid Chromatography (VLC)-fractionated ethyl acetate extracts of leaves of *M. cordata* have anticancer effects. *M. cordata* VLC fractions 6, 7, 10 and 11 were found to significantly inhibit MCF-7 cell proliferation at levels statistically similar to important breast cancer drug doxorubicin. Inhibition was in a dosage-dependent manner and at 25 μg mL^{-1}, *M. cordata* fractions 6 and 7 began to inhibit cell proliferation more than doxorubicin, suggesting that these fractions become more potent than doxorubicin at higher concentrations. This reports a remarkable find with great potential for the cancer drug discovery program.

After establishing anticancer effects, the study sought to study its possible mechanisms of action through apoptosis and oxidation and to evaluate each fraction for cytotoxicity to normal cells using J774A.1 mouse macrophage cell line and brine shrimp lethality assay. It found that the identified fractions exert their anticancer activities through different mechanisms. Fractions also differed in the specificity of their cytotoxicity.

Mikania cordata fraction 6 exerted anticancer effects through both apoptotic and oxidative pathways. The TUNEL assay showed the occurrence of DNA fragmentation in the MCF-7 cells treated with fraction 6. The DPPH assay also showed oxidation of the 1,1-diphenyl-2-picrylhydrazyl reagent treated with fraction 6. This fraction, however, exerts non-specific cytotoxicity, inhibiting proliferation of both normal and cancer cells, as shown in cytotoxicity estimates to normal J774A.1 murine macrophages and toxicity estimates to brine shrimps.

Mikania cordata fractions 10 and 11 show very interesting finds. They exert cytotoxicity more specific for cancer cells. They inhibited proliferation of the MCF-7 cells more than the J774A.1 cells and exhibited reduced mortality to brine shrimps.

Many chemotherapeutic regimens have been problematic due to their cytotoxicity to normal cells which cause debilitating side effects in patients that use them. This can be mitigated by the use of chemicals that have more specific cytotoxicity for only the cancer cells. Such treatments can improve clinical outcomes, since, clinicians can give higher concentrations for longer periods of time, without significantly affecting quality of life of the patients.

The mechanism by which fractions 10 and 11 exert their cancer-specific cytotoxicity, however, is not mediated through either apoptotic or oxidative pathways. Studying their mechanism of action could therefore, elucidate pathways for targeted therapy.

Mikania cordata fraction 7, though showing non-specific cytotoxicity, also exerts anticancer effects through pathways other than apoptosis and oxidation. Such findings become significant in light of the current problem of resistance. Breast cancer cells have been able to develop resistance to current regimens that work by common mechanisms. Novel mechanisms of action could therefore, be potential treatments for resistant breast cancers.

The phytochemical screening pointed to terpenes and saponins as possible candidates responsible for exerting anticancer activities. On a further TLC analysis, the terpenes and saponins present in each fraction were found to be different in polarity which points to the presence of many, not just a single, active component in *Mikania cordata*, that is able to exert anticancer effects. Further dissection and analysis of individual components could lead to purified chemotherapeutic drugs with increased potency and novel mechanisms of action.

The results from the present study clearly validate the anticancer potential of *Mikania cordata* extract. The findings become very important in light of the problem of current chemotherapeutic regimens being very expensive. *Mikania cordata* is a widely available plant, that is typically treated as unwanted weeds, even being included in the compendium of world's worst weeds. The study shows that the weed could be a potential cheap source of treatment for cancer patients.

In conclusion, *Mikania cordata* shows anti-cancer effects in MCF-7 human breast adenocarcinoma cells. *Mikania cordata* ethanol extract-vacuum liquid chromatography fractions 6, 7, 10 and 11 inhibited proliferation of MCF-7 breast cancer cells. The anticancer effects of each fraction act through different mechanisms of action and differ in specificity of cytotoxicity. *Mikania cordata* fraction 6 has non-specific cytotoxicity and acts through apoptosis and oxidative pathways. *Mikania cordata* fraction 7 also exerts non-specific cytotoxicity but acts through mechanisms other than apoptosis and oxidation. *Mikania cordata* fractions 10 and 11 exert cytotoxicity more specific for cancer cells and acts through mechanisms other than apoptosis and oxidation. Phytochemical screening identified terpenes and saponins in the fractions that could be candidates responsible for the anti-cancer activity.

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