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Phytochemical Screening and Synergistic Anti-proliferative Activity against Selected Cancer Cell Lines of *Moringa oleifera* and *Indigofera arrecta* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JWN, EA, DKN, RM, MJ, RWM, JC, CMN and PM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JWN, EA and PM managed the analyses of the study. Author JWN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants present a plausible source for anticancer agents. Combination of plant extracts and plant-derived compounds with the currently used cancer drugs has shown a marked improvement of the conventional drugs' efficacy and reduced toxicity. This study evaluated; phytochemical screening, antiproliferative activity and drug interaction potentials of *Moringa oleifera* and *Indigofera arrecta* leaf extracts with 5-fluoro uracil against selected cancer cell lines. Phytochemical screening was done using standard procedures. The common 3– (4, 5-dimethylthiazol-2-yr) -2, 5-diphenyltetrazolium (MTT) assay was used to determine the growth inhibitory potential of the extracts towards cancer cells. Drug interaction assays were done using constant ratio combination method. Alkaloids, terpenoids, tannins, flavonoids, glycosides, phenols and saponins were found to be present in the plant's extracts. *M. oleifera* and *I. arrecta* methanol-dichloromethane extracts had the highest activity compared to water extracts. All the extracts showed antiproliferative activities towards; HCC 1395 (breast), DU145 (prostate) and Hela (cervical) cancer cell lines. The extracts were not cytotoxic towards Vero cells (IC_{50}>1000 µg/ml). *I. arrecta and M. oleifera* inhibited DU145 the most with IC_{50} values of 111.110 µg/ml and 66.290 µg/ml respectively. The plant extracts synergistically inhibited the growth of cancer cells (CI<1). Combination of the plant extracts and 5-Fluorouracil depicted that the concentration of the conventional drug could be reduced and yet achieve the same desired effect against cancerous cells (Dose reduction index (DRI) >1). Further studies to isolate the bioactive compounds and deduce the probable mechanisms of action are recommended.

Keywords: Anti-proliferation; cytotoxicity; phytochemical; Moringa oleifera; Indigofera arrecta.

1. INTRODUCTION

Medicinal plants are used in different countries in the treatment of various diseases. Approximately, 80% of the world's population relies on traditional medicine for their primary healthcare needs [1]. Medicinal plants which are believed to be safe, cheap and readily available have been used for centuries in the treatment of various diseases affecting human beings including cancer [2]. *Moringa oleifera (*commonly known as the drumstick tree) is a plant from Moringaceae family. In Kenya, it is widely distributed in Laikipia, Baringo, Kilifi and Makueni counties. It is a shrubby deciduous tree [3], growing to a height of 10-12 m. The plant is rich in vitamin A, B, C, D, E and K [4] and has been employed in the treatment of many diseases traditionally such as hysteria, scurvy, prostate problems and bladder discomfort. Various studies have revealed a wide array of medicinal properties of the plant i.e. anti-inflammatory, antioxidant [5,6], strengthening of the immune system and wound healing [7]. Moringa seeds are effective against skin-infectious bacteria [8], while the roots and bark are used to relieve cardiac and circulatory problems [9]. The leaves have high iron content thus used in anaemia treatment [10]. In addition, various Moringa plant parts have wide uses; the flowers can be used as a beverage. Gum from the stem in calico printing and also seed kernels in asthma treatment [11].

I. arrecta is a plant in the *Fabaceae* family and is majorly found in tropical regions. *I. arrecta* is cultivated for its dye, especially in India and the leaves are traditionally used in the treatment of epilepsy, nervous disorder, ulcers and diabetes mellitus [12]. The leaves also contain antibacterial activities [13] with the potential to heal sores.

Despite the existing wealth of knowledge on the medicinal potential of these two plants, their anticancer activity needs to be elucidated. This study tested the antiproliferative activity of the plant's extracts against selected cancerous and normal cells. The study further evaluated the synergistic effects of *I. arrecta* and *M. oleifera* extracts with 5-Fluorouracil, an antimetabolite and anti-cancer chemotherapeutic drug that is widely used in the treatment of colon, prostate, breast cancer. The drug 5-Fluorouracil is an analogue of uracil which rapidly enters the cell and is converted to fluorodeoxyuridine monophosphate and fluorodeoxyuridine triphosphate inside the cell, the active metabolites of 5-Fluorouracil [14]. These active metabolites disrupt the transcription process and interfere with thymidylate synthase, an enzyme involved in nucleotide synthesis [14]. Despite its wide application in cancer treatment, a number of side effects have been observed in cancer patients under 5-Fluorouracil treatment including hair loss, nausea and vomiting, loss of appetite

and diarrhoea [15]. It is therefore important to search for alternative therapeutic agents with fewer side effects.

Plant extracts contain a plethora of compounds which act concertedly in the treatment of various diseases including cancer, with the potential of interaction with prescribed pharmaceutical drugs to improve (synergistic) or decrease (antagonism) the therapeutic effect [16]. The uses of *M. oleifera* and *I. arrecta* compounds as a commercial medicine for a chemopreventive agent have not been developed yet. However, *M. oleifera* showed a positive synergistic effect in increasing cytotoxicity of doxorubicin against cervical cancer cell lines (Hela) [17]. The combination of doxorubicin and *M. oleifera* resulted in enhanced apoptosis induction although the mechanism of action involved by combining the two drugs is still unclear. Previous studies have suggested that combining plant extracts with available cancer drug therapy, could reduce the toxic side effects of the drugs as well as enhance the chemo-preventive effect of the drugs [18,19]. *M. oleifera* extracts have long been used in traditional medicine [18,19] which proof the plant is safe to consume. Despite the fact that a lot of probable pharmacological uses have been reported, studies on the effect of the concurrent use of the plant with other medicinal plants are lacking. This study reports antiproliferative potential of *M. oleifera* and *I. arrecta* leaf extracts towards selected cancer cell lines.

2. MATERIALS AND METHODS

2.1 Plant Collection

The leaves of *M. oleifera* were collected in Makueni County while those of *I. arrecta* in Kiambu County in Kenya with the aid of local traditional health practitioners and identified by a botanist. Botanical samples (Voucher numbers JWN/MO/01/2016 & JWN/IA/02/2016 respectively) are kept at CTMDR, KEMRI. The samples were cleaned under running water to remove dust particles and other particulates and dried at room temperature. The dried leaves were grounded into a fine powder using Gibbons electric grinding machine (Wood-Rolfe Road, Tollesbury Essex, UK).

2.2 Preparation of the Crude Extracts

The organic solvent extraction was done according to a method by [20] with slight modifications. A beam balance was used to weigh 150 g of the powdered plant material and placed in a flat-bottomed conical flask, submerged using a mixture of methanol and dichloromethane in the ratio 1:1 with frequent agitation. Extraction was allowed to proceed for 24 hrs, followed by filtration using Butcher funnel and Whatman No. 1 filter paper. The samples were re-soaked for 24 hrs and the filtrates concentrated using Buchi water bath 8-480 rotary evaporator (Buchi Laboratech N IK AG, Switzerland) at 40°C. The concentrated samples were weighed and stored at 4°C until use.

The aqueous extract of each of the plants was prepared by soaking 100 g of the powdered plant into a 500 ml of distilled water and heated at 60°C in a water bath for 6hrs. It was covered and left to cool at room temperature. The material was then filtered through muslin gauze and filtrate frozen for 24 hrs. The filtrate was then lyophilized using Modulyo Edwards freeze drying machine (Edwards High Vacuum, Crawley, England and Britain). The lyophilized samples were then weighed in air tight bottles at -20°C until used [21].

2.3 Preliminary Phytochemical Screening

The chemical test was carried out on the plant extracts using standard procedures to identify pharmacologically important constituents as described by [22].

2.4 Test for Total Phenols

2 ml of the crude extract was put in a test tube. Two drops of 2% $FeCl_2$was then added. A blue, red, green or purple colouration depicts the presence of phenols.

2.5 Test for Steroids

2.5.1 Liebermann burchard reaction

2 ml of each crude extract was put in different test tubes and 5ml of chloroform added. The samples were filtered separately then mixed with 2 ml of acetic acid. Concentrated sulphuric acid was then added along the side of each test tube. Observation of blue-green ring indicates the presence of steroids.

2.6 Test for Glycosides

0.5 ml of each crude extract was put in a test tube and 2 ml of chloroform added and mixed. 2

ml of concentrated sulphuric acid was then carefully added and the contents mixed. A red-brown colour portrays the presence of steroidal ring (glycine portion of glycosides).

2.7 Test for Alkaloids

2 ml of each crude extract was put in a test tube, followed by addition of 1% hydrochloric acid and the mixture heated gently. A few drops of Mayer's reagent were added and observations made. Formation of a precipitate delineates presence of alkaloids.

2.8 Test for Flavonoids

2 ml of each crude extract was put in a test tube and 5ml of dilute ammonia added. 2 ml concentrated sulphuric acid was then added to the mixture of each test tube and shaken. Presence of a yellow colour indicates the presence of flavonoids.

2.9 Test for Terpenoids

2 ml of each crude extract was put in a test tube. 2 ml of chloroform was then added and the mixture vortexed. The samples were then evaporated to dryness and about 2 ml of concentrated sulphuric acid added in each test tube and heating done for 2 minutes. A greyish colour indicates the presence of terpenoids.

2.10 Test for Saponins

5 ml of each crude extract was put in a test tube and 5 ml of water added. The contents were then mixed. Observation of a stable form shows the presence of saponins.

2.11 Test for Tannins

2 ml of each crude extract was added to the test tubes and the contents heated to boil. About 1% of $FeCl_3$ was then added drop wise in each test tube and observations made. A brownish colouration indicates the presence of tannins.

2.12 Antiproliferative Assay

2.12.1 Sample preparation

Briefly, 10 mg of the plant extracts in a 1.5 ml Eppendorf tube was weighed using an analytical balance and 100 µl dimethylsulfoxide (DMSO) solution added and the mixture vortexed. 100 µl of this mixture was then transferred into another 1.5 ml Eppendorf tube and topped up with 900 µl Phosphate Buffer Saline (PBS) to make a concentration of 1,000 µg/ml. This was then stored at -20°C until use.

2.12.2 Cell line culturing

Vero cells (normal) and selected cancer cells (American Type Culture Collection: ATCC) were provided by Kenya medical research institute (KEMRI), Center for Traditional Medicine and Drug Research (CTMDR). The cells were thawed in a water bath at 37°C and cultured inT-75 flasks with Minimum Essential Medium (MEM, SIGMA USA) supplemented with 10% Fetal Bovine Serum (FBS) and 100 µg/ml streptomycin then incubated at 5% CO_2 at 37°C for 72 hrs to attain confluence.

2.12.3 Antiproliferative assay

Upon attainment of 70-100% confluence, the growing cells were washed using phosphate buffer saline and harvested by trypsinization. The number of viable cells was determined using Trypan blue exclusion method (cell density counting) using a hemocytometer. An aliquot of 100 µl at a density of 2.0×10^4 cells/well was seeded into 96-well micro plates and incubated at 37°C for 24 hrs at 5% CO_2. After 24hrs incubation, 15 µl of the test samples were added at seven different concentrations each serially diluted at 1000 µg/ml, 333.33 µg/ml and 111.11 µg/ml 37.04 µg/ml, 12.35 µg/ml, 4.12 µg/ml, 1.37 µg/ml starting from row H to B. Row A served as cell control. The plates were then incubated at 37°C in 5% CO_2 for 48 hrs. The viability of the cells after extract addition and incubation was done using MTT assay. The effect of the test samples on the cells was quantified by the ability of the living cells to reduce the yellow 3- (4-5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) to a purple product called formazan [23]. After 48 hrs, 100 µl of the medium was aspirated and 10 µl of the MTT solution added to the remaining medium (100 µl) in each well and incubated for 4hrs in 5% CO_2 at 37°C. The overlaying media was then removed from the plates and the formazan crystals dissolved using 50 µl of 100% DMSO. The wells contents were thoroughly shaken followed by absorbance reading using a calorimetric reader at 562 nm and a reference wavelength at 690 nm respectively. The percentage cell viability at different

extracts concentration was calculated using the formula:

Percentage cell viability= [100-(At-Ab)/ (Ac-Ab)] ×100

Where:

A_t = Absorbance value of test compound.
A_b =Absorbance value of blank
A_c = Absorbance value of control

The effect of *Moringa oleifera* and *Indigofera arrecta* extracts on Vero cells was expressed in CC_{50} values (the extracts concentration which kills 50% of the treated cells) [24].

2.12.4 Evaluation of drug interaction by chou-talalay method

Drug synergy was determined by isobologram and combination index method was derived from median effect principle of Chou and Talalay using compuysn software. The IC_{50} values obtained from inhibitory experiments were used in the drug interaction assays. A fixed ratio combination technique was used in mixing of the samples based on relative potency using the formula: $EC50_{(a)}$ /$EC50_{(b)}$. Where$EC50_{(a)}$ and $EC50_{(b)}$ represents the effective cytotoxic doses of drug A and drug B respectively that is estimated to kill 50% of the cells. Drug A is more potent than drug B. The drug interaction effect was presented in combination index (CI) values. Combination index is a mathematical and quantitative representation method of two drug pharmacologic interactions using data from the growth inhibitory experiment. Combination index values were calculated according to the equation: CI= $[(D_1/E_1) + (D_2/E_2)]$ using the computer software.

Where D_1 and D_2 are the actual drug doses used in the combination during dosing experiments and E_1 and E_2 is the theoretical individual drugs level that would be expected to achieve the experimentally measured response or the iso-effective doses. E_1 and E_2 can be calculated using Dm and m values from median effect equation, $fa/fu= (D/Dm)^m$. Where fa is the fraction of cell population affected and fu is the fraction of cell population unaffected, D is a dose of the drug given, Dm is the median effect dose and m is the slope of the curve.

Combination index values were interpreted using the Chou and Talalay referencing were CI between 0.9-1.1 was said to be additive, CI between 0.85-0.90 slightly synergistic, moderate synergistic when CI=0.7-0.85, synergistic for CI between 0.3-0.7, 0.1-0.3 strong synergy and CI <0.1 highly synergistic. Combinations of samples with CI values between 1.1-1.2 were interpreted to be slightly antagonistic, moderate antagonistic for CI values between 1.20-1.45, antagonistic for CI values between 1.45-3.3, strong antagonism for CI values between 3.3-10, very strong antagonism for CI>10 and additive if CI=1 [25].

2.12.5 Data management, analysis and presentation

All activities undertaken were recorded in a laboratory note book. Analysis of statistical data obtained from this study was done using excel data sheet and Statistical Package of Social Science (SPSS Version 20). The difference between the treatment and the control was tested for statistical significance using One-way Analysis of Variance (ANOVA) ($p \leq 0.01$). The IC_{50} and CC_{50} values were expressed as Mean ± Standard Error of Mean (SEM). Compusyn software was used in the analysis of the drugs interaction effect. Tables were used in clear presentation of the results.

3. RESULTS

Table 1 shows the phytochemical compositions of *I. arrecta* and *M. oleifera* water and methanol: dichloromethane leaf extracts. On the other hand, Table 2 shows the IC_{50} and the selectivity index of the tested successive extracts against selected cancerous cells.

The results obtained in Table 1 indicate that alkaloids, terpenoids, flavonoids, glycoside, phenols and tannins were present in the water and dichloromethane: methanol leaf extracts of *M. oleifera* and *I. arrecta*. However, it is important to note that saponins were absent in both extracts of *M. oleifera* while terpenoids were absent in *I. arrecta* extracts. The presence of these phytochemical could be implicated in the medicinal value of the two plants. In comparison, phytocompounds such as flavonoids, alkaloids, saponins and terpenoids have been shown to be potent anticancer agents, giving rise to numerous convectional drugs, which are used in mainstream medicine and in the primary therapeutic strategies for numerous conditions including cancer [26].

The selectivity index ranged between 1.493±0.999 µg/ml and 473.569±12.083 µg/ml.

Plant extracts having SI values greater than 3 were considered to be highly selective to the specific cancer cells. The methanol and dichloromethane extracts were highly selective against DU145 cancerous cells, with a selectivity index of 11.427±1.061 µg/ml for *M. oleifera* and 4.975±2.892 µg/ml for *I. arrecta*. In the cytotoxicity studies, all extracts had IC_{50}> 500 µg/ml. The CC_{50} value of Vero cell ranged between 418.257±8.735 µg/ml and 997.667±1.453 µg/ml. The extracts gave an IC_{50} that was significantly different from 5-Fluorouracil (P≤0.05).

Table 1. Phytochemical composition of the *Indigofera arrecta* and *Moringa oleifera* leaf extracts

Phytochemical	MOMD extracts	MO water extracts	IAMD extracts	IA water extracts
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Terpenoids	+	+	-	-
Saponins	-	-	+	+
Glycosides	+	+	+	+

Key: - (Absent), + (Present), MO-Moringa oleifera, MOMD- Moringa oleifera methanol dichloromethane extract. IA- Indigofera arrecta, IAMD- Indigofera arrecta methanol dichloromethane extract

Table 2. Selectivity indexes of *Indigofera arrecta, Moringa oleifera* leaf extracts and 5-Fluorouracil drug in µg/ml

Extracts	Selectivity index: Vero/ Du145	Selectivity index: Vero/ Hela	Selectivity index: Vero/ HCC1395
MO MD	11.430±1.060	2.530± 0.820	1.900± 0.790
MO water	1.560± 0.640	1.490± 0.990	1.500± 0.870
IA water	8.160± 0.570	2.880± 0.560	3.130± 0.820
IA MD	4.97±2.890	166.450±2.420	1.980±0.550
5-Fluorouracil	473.570±12.080	3.560± 0.500	134.140±5.070

Key: MO- Moringa oleifera, IA- Indigofera arrecta, MD-methanol dichloromethane extract

Table 3. Turkey's multiple comparisons of the IC_{50} values of different selected cell lines that were treated with the plant extracts and the convectional drug (5-fluorouracil)

Comparisons	P- value
DU145 Moringa MD Versus DU145 5-Fluorouracil	0.001[a]
DU145 Moringa MD Versus DU145 5-Fluorouracil	0.001[a]
DU145 Indigofera MD Versus DU145 5-Fluorouracil	0.001[a]
DU145 Indigofera water Versus DU145 5-Fluorouracil	0.001[a]
HCC1395 Moringa MD Versus HCC1395 5-Fluorouracil	0.085[b]
HCC1395 Moringa water Versus HCC1395 5-Fluorouracil	0.012[a]
HCC1395 Indigofera MD Versus HCC1395 5-Fluorouracil	0.002[a]
HCC1395 Indigofera water Versus HCC1395 5-Fluorouracil	0.560[b]
Hela Moringa MD Versus Hela 5-Fluorouracil	0.598[b]
Hela Moringa water Versus Hela 5-Fluorouracil	0.001a
Hela Indigofera MD Versus Hela 5-Fluorouracil	1.000[b]
Hela Indigofera water Versus Hela 5-Fluorouracil	0.042[a]
Vero Moringa MD versus Vero 5-Fluorouracil	0.085[b]
Vero Moringa water Versus Vero 5-Fluorouracil	0.012[a]
Vero Indigofera MD Versus Vero 5-Fluorouracil	0.002[a]
Vero Indigofera water Versus Vero 5-Fluorouracil	0.560[b]

Key: a-Significant at P≤0.05, b-Not significant at P≤0.05, Hela-Cervical cancer cell lines, DU145- Prostate cancer cell lines, HCC1395- Breast cancer lines, Vero- Normal monkey kidney cells

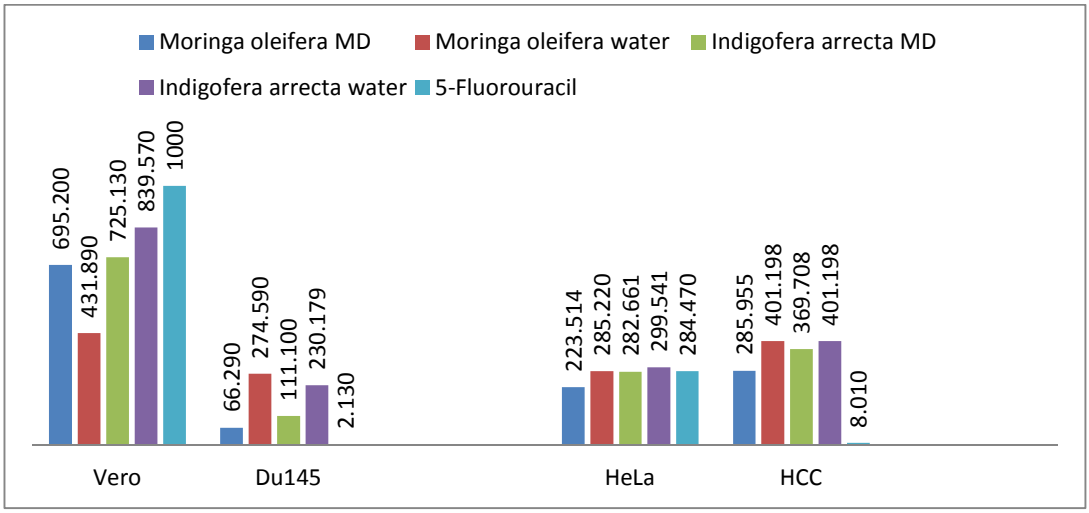

Fig. 1. Effects of plant extracts on selected cancerous and normal cells in µg/ml

Key: DU145-Prostate cancer, Vero cell- Normal monkey kidney cell, HCC1395-Breast cancer, Hela-Cervical cancer, MD- Methanol: dichloromethane

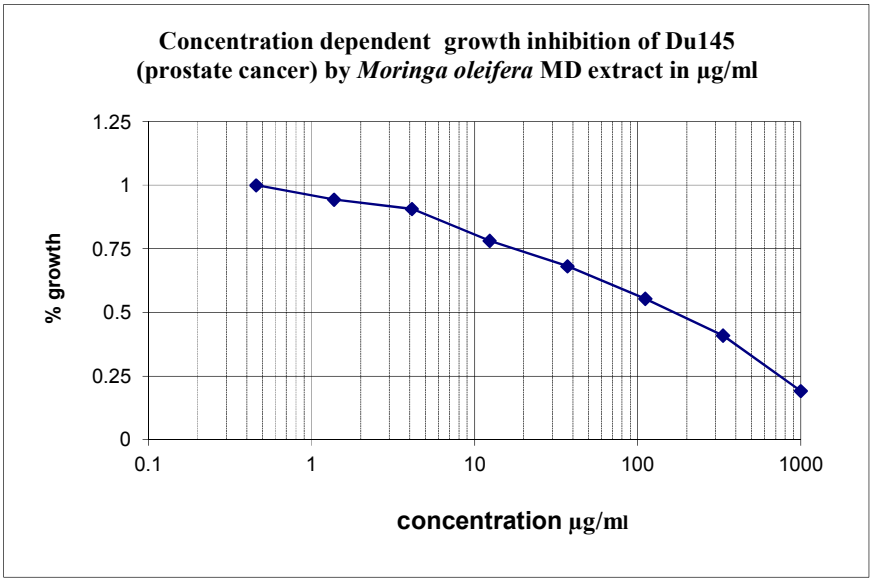

Fig. 2. Shows the growth inhibition percentage of cancer cells against the concentration of the extracts. The plant extracts inhibited the growth of the selected cancerous cells in a concentration-dependent manner

The graph above shows that methanol dichloromethane leaf extracts had greater activity compared to water extracts, this is because methanol dichloromethane extracts both polar and non-polar compounds responsible for the observed a higher degree of activity of the extracts on the selected cell lines compared to water which extracts only the polar compounds and some impurities. *M. oleifera* and *I. arrecta* methanol dichloromethane leaf extracts had higher inhibitory growth effect towards DU145 cancer cells with an IC_{50} value of 66.29 µg/ml and 111.10 µg/ml respectively.

Anticancer potential of *M. oleifera* leaves extracts have been shown to inhibit the growth of colorectal, breast, B16F10 and pancreatic cancer cells [27]. However, this study reports antiproliferative potential of *M. oleifera* extracts growth inhibition against Hela (cervical) and HCC1395 (breast) cancer cells. Additionally, this study reports for the first time to the best of our

knowledge, the antiproliferative potential of *I. arrecta* leaf extracts against Hela, DU145 and HCC1395 cancerous cells.

The samples interaction potential was analyzed using the Compuysn software. The shift of combination curves below the most active single drug shows a synergistic effect. All combinations (*M. oleifera* and *I. arrecta*, *M. oleifera* and 5-Fluorouracil, *I. arrecta* and 5-fluoro uracil) depicted synergistic effect on the various cell lines tested (CI<1). *I. arrecta* with 5-Fluorouracil and *M. oleifera* with 5-Fluorouracil combinations on HCC1395 and on DU145 demonstrated the strong synergistic effect (CI=0.1-0.3). In both Figs. 3 and 4, *Indigofera arrecta* extracts in combination with 5-Fluorouracil demonstrated a stronger synergistic effect compared to *Moringa Oleifera* combined with 5-Fluorouracil. This may be due to the difference in modes of action of

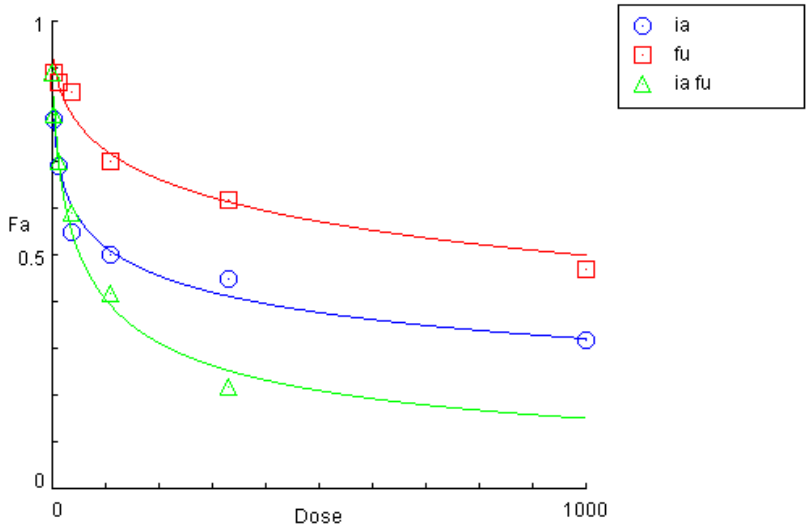

Fig. 3. Combination effect plots of *Indigofera arrecta* extract and 5-Fluorouracil on DU145 (prostate cancer cells) in µg/ml

Key: a- Indigofera arrecta methanol dichloromethane extract, fu-5-Fluorouracil, a fu- a combination of Indigofera arrecta and 5-Fluorouracil, Fa- a fraction of dead cells

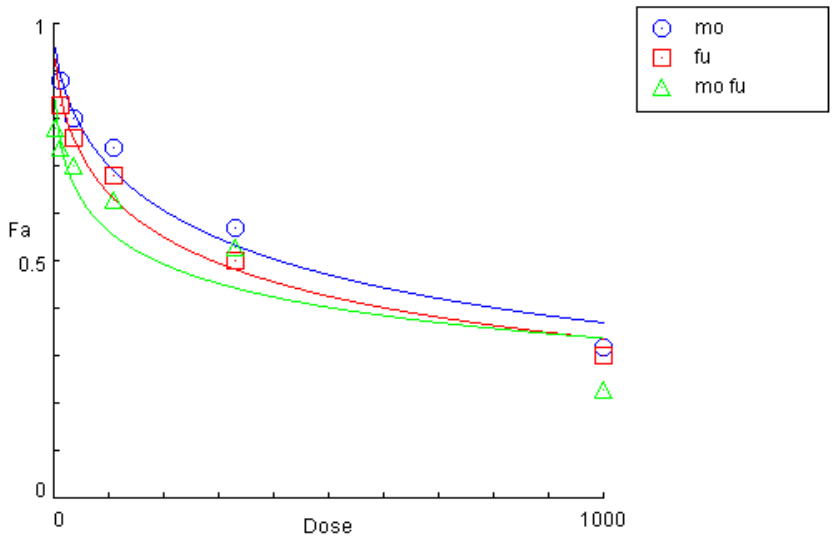

Fig. 4. Combination of *Indigofera arrecta* and 5-fluorouracil on HCC cancerous cells µg/ml

Key: fu- 5-Fluorouracil, mo- Moringa oleifera, of- Moringa oleifera and 5-Fluorouracil combination, Fa- a fraction of dead cells, Dose- dose in µg/ml

various compounds present in the extracts. This could also be attributed to the differences in "shutting down" of various cancer survival pathways such as NF-Kβ signalling cascade in the extracts. The 0.5 fa and 0.7 fa indicates 50% and 70% cell inhibition levels respectively. A combination index<1 indicates synergistic effect. The combination index at 0.5fa and 0.7 fa (fraction of cell affected) for *M. oleifera* and 5-Fluorouracil was 0.58282 µg/ml and 0.27011 µg/ml. That of *I. arrecta* and 5-Fluoro uracil at 0.5 fas and 0.7fa was 0.2357 µg/ml and 0.4963 µg/ml, while the combination index of *M. oleifera* and *I. arrecta* at 0.5 and 0.7 fa was 0.14891 µg/ml and 0.0478 µg/ml. This provides evidence of the superiority of the drug combination compared to its single agents.

4. DISCUSSION

M. oleifera possesses numerous compounds with excellent health benefits including anti-oxidant and anti-cancer properties [28]. *M. oleifera* have been widely used traditionally to treat various ailments such as cancer [29]. This is the first study to show possible synergistic anti-proliferative activities of *M. oleifera* and *I. arrecta* leaf extracts on selected cancer cell lines. The phytoconstituents of *M. oleifera* were shown to possess antiproliferative effects on various cell lines in comparison to previous studies, *M. oleifera* leaves have been reported to contain glucosinolates, isothiocyanates, niazimicin, niaziminin and quercetin which have also attributed to the anticancer effect [30]. A recent study has also shown the significance of *M. oleifera* phytochemicals in prostate cancer therapy [31]. The phytochemicals in *M. oleifera* and *I. arrecta* leaf extracts enhanced the activity of selected cancer cell lines that possess less toxicity, thus showing its potential as a potent and safe natural agent in cancer therapy and drug design. Similarly, these active compounds in *M. oleifera* have been shown to act as anticancer agents in lung cancer by inducing cellular apoptosis and subsequent cell death [32]. The plant has been shown to exhibits anti-cancer potential by interfering with the signal transduction cascade that promotes cancer cell proliferation and progression [33]. According to our studies, we detected a remarkable decrease in viable cells on ELIZA cells analyzer. The cell survival was significantly reduced in the presence of these extracts as compared with the corresponding control (Figs. 1 and 3). The decrease in cell number can be attributed to the phytochemical compounds found in the extracts which promote the inhibition of cancer survival protein expression. This study also shows that synergistic anti-proliferative activity of the two extracts with 5-Fluorouracil significantly increased activity compared to using the extract singly (Figs. 3 and 4). The cell motility influenced by the synergy of the two extracts gave additional information about the inhibitory role of *M. oleifera* and *I. arrecta* by reducing the cell motility phenomenon of selected cancer cell lines. The overall mechanism of reduced cell viability and active participation in normal cellular activity could also be attributed to the "shutting down" of various cancer survival pathways including the NF-Kβ signalling cascade by down regulating the important component p65 of NF-kβ by the compounds present in Moringa extracts [34]. Our results agree with the reports obtained on leaves extract as discussed above. These findings add to the growing evidence supporting the promising role of *M. oleifera* as an anti-cancer agent and open a new vista for synergistic anti-proliferative activities of *M. oleifera* and *I. arrecta* leaf extracts. The two extracts could represent a valuable therapeutic tool for use as part of a therapy for the treatment of breast, prostate and cervical cancer cell.

5. CONCLUSION

The study validates anti-proliferative and synergistic activities of *M. oleifera* and *I. arrecta* leaf extracts towards cancer cells. The activity observed can be attributed to the presence of the pharmacologically important group of phytochemicals present in the plant extracts. *M. oleifera* and *I. arrecta* leaf extracts selectively inhibited the growth of the cancerous cells. This authenticates the traditional application of *M. oleifera* and *I. arrecta* leaf extracts towards cancer treatment and management.

The combination of the two extracts with 5-Fluorouracil showed improved antiproliferative activities compared to single extracts. This indicates a probable use of *M. oleifera* and *I. arrecta* with 5-Fluorouracil in cancer treatment and management.

Further studies are needed to isolate the bioactive compounds from the crude extracts which could be used as makers in the standardization of formulations from the two plants for cancer treatment and management. *In-vivo* studies which could better predict the potential application of the plant extracts and their bioactive compounds in chemotherapy are

proposed. Elucidation of the mechanism of action of the plant extracts and pharmacologically active compounds is recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was sought from Kenya Medical Research Institute (KEMRI); Scientific and Ethics Review Unit (SERU) before conducting the study (Approval number KEMRI/SERU/ CTMDR/ CSCP034/ 3341).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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