

# ***In vitro* Antiproliferative Studies of Selected Medicinal Plants on Cancerous and Normal Cells**

## **ABSTRACT**

Globally, approximately 13% of all deaths annually are attributed to cancer. Surgery, radiation and chemotherapy are the current treatment techniques for cancer; however, these methods are expensive, have high failure rates and have been associated with detrimental side effects. Plant derived products could be good candidates in alleviating challenges being experienced with these current methods. This study aimed at evaluating the phytochemistry, antiproliferation potential, and probable mechanism of action of *Albizia gummifera*, *Rhamnus staddo* and *Senna didymobotrya* plant extracts. The 3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium (MTT) assay dye was used in the determination of the antiproliferative activity of the extracts. Extracts induction potential of p53 (apoptosis) and VEGF (angiogenesis) genes' expression was evaluated using Real Time PCR. Phytochemical screening was done as per standard procedures. Several plant extracts exhibited antiproliferative activity against the cancerous cell lines tested showing selective toxicity to cancer cells while sparing the normal cells (SI  $\geq$  3). An upregulation of p53 and down-regulation VEGF genes was observed. Phytochemical screening revealed presence of pharmacologically important phytochemicals in the plant's extracts. The study findings suggest exploitation of these plant extracts as potential candidates for development of drugs for the management of breast and prostate cancer.

**Keywords:** *Albizia gummifera*; *Rhamnus staddo*; *Senna didymobotrya*; antiproliferative; cancer; p53; VEGF.

## **1. INTRODUCTION**

Cancer is among the leading causes of morbidity and mortality worldwide. There were an estimated 19.3 million new cases of cancer and nearly 10 million deaths from cancer worldwide in 2020 [1]. The factors that have been associated with high cancer risk include high body mass index, low fruit and vegetable intake, lack of physical activities, environmental pollution, tobacco use and alcohol [2]. Chemotherapy, radiation, surgery and hormonal and targeted therapy are the main strategies employed in the management of cancer. Despite their effectiveness, they lack specificity and have various side effects such as hair loss, peripheral neuropathy and cardiac damage among others [3]. Due to these challenges, people have turned to the use of medicinal plants as alternative therapies because they are thought to be cheap, effective, safe and easily accessible. It has been estimated that over 30% of the plant's species contain secondary metabolites which are useful in treatment of various diseases such as cancer [4]. The use of naturally derived products from medicinal plants that selectively induce apoptosis and reduce angiogenesis could serve as an

alternative to the current cancer treatment regimens [3].

Several important bioactive compounds that produce desirable physiological activities have been derived from plants. These compounds could serve as new leads and clues for modern drug design [5]. These important bioactive constituents of plants include but are not limited to alkaloids, tannins, flavonoids, terpenoids and phenolic compounds [6]. During the synthesis of compounds with specific activities to treat various diseases such as cancer, it is important to know the correlation between the phytoconstituents and the bioactivity of plants [7].

The determination of the molecular mechanisms underlying neoplastic transformation and progression have resulted in the understanding of cancer as a genetic disease, which evolves from the accumulation of a series of acquired genetic lesions [8]. Protein 53 (p53) is a tumor suppressor that eliminates and inhibits multiplication of abnormal cells through induction of apoptosis [9]. It is one of the key orchestrators of cell signaling pathways related to apoptosis and cell cycle, which have an essential role in the development and progression of complex

diseases such as cancer. Studies have shown that medicinal plants can activate apoptotic genes [10].

Angiogenesis is a key process in cancer promotion. It is an important pathological event associated with tumor growth and metastasis. Vascular Endothelial Growth Factor (VEGF) plays an important role in this event [11]. It is a physiological process of formation of new blood vessels on already existing ones. The newly formed blood vessels facilitate the metastatic dissemination of cancer cells. In most cancers, angiogenesis correlates with disease stage and metastasis [12]. Various reports have shown that plant extracts and plant derived compounds have the potential to down regulate VEGF [13]. This study therefore aims at evaluating the antiproliferative activity of *S. didymobotrya*, *A. gummifera* and *R. staddo* MeOH: DCM and aqueous plant extracts and their probable mechanism of action in cancer growth inhibition.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials Used

The plant parts of *A. gummifera*, *R. staddo* and *S. didymobotrya* were used in this study. *A. gummifera* (JF Gmel.) C.A. Sm. belongs to the family Mimosoideae. The plant is known by different names in Swahili such as Mshai, Mkenge and Mchai mbao, and peacock flower in English. *R. staddo* A. Rich. belongs to family Rhamnaceae. It is commonly known as staddo or buckthorn and commonly referred by the Tugen community in Baringo County, Kenya as Ng'oliny. *S. didymobotrya* (Fresen.) Irwin & Barnebey belongs to the family Caesalpinaceae. It is a 30-90 cm tall small tree or a several stemmed shrub. It is locally known as Mwinu in the Kikuyu, Meru and Embu community and Muumai in Kamba community.

The leaf, stem bark and root bark of *S. didymobotrya* and *R. staddo* were collected from Laikipia County; 0.0196463N (Latitude), 37.0837843E (Longitude). *A. gummifera* plant parts were collected from Ngong Forest, Kajiado County; 1.355676N (Latitude), 36.664274E (Longitude). Harvesting was done sustainably. Identification of the botanical samples was conducted by a qualified botanist and voucher specimens (RAM 2017/01, RAM 2017/03 and RAM 2017/2 respectively) stored at the University of Nairobi Herbarium.

The plant samples collected were dried at room temperature and then ground into fine powder using Gibbons electric mill (Wood Rolfe Road Tolles Bury Essex, UK). The ground samples were then stored in air tight bags at room temperature until use.

### 2.2 Extraction

#### 2.2.1 Aqueous extraction

About 200 g of each sample was weighed and submerged in 1litre of double distilled water. Extraction was done in an aqueous bath at 60°C for 2 hrs. After cooling, the extract was decanted in a clean 1000ml conical flask and filtered using a Whatman No. 1 filter paper. The filtrate was then freeze dried using a freeze dryer (Modulyo Edwards high vacuum, Crawley England, Britain, Serial No. 2261). The extract was weighed and stored at 4°C in air tight vials until use.

#### 2.2.2 Organic extraction

Briefly, 200 g of each sample was weighed, put in a flat-bottomed conical flask and solvent added to cover the sample completely and left to stand for 24 hrs. A Whatman No. 1 filter paper was used to filter and the sample re-soaked again for 24hrs. Extraction was done using methanol: dichloromethane (1:1). The solvents were removed using a rotary evaporator (Büchi, Switzerland) and the concentrated extracts packed in air tight vials and stored at 4°C until use.

### 2.3 Cell Culturing

DU 145 (prostate cancer), HCC 1395 (breast cancer) and Vero E6 (normal) cells obtained from ATCC (Manassas, VA, USA) were used. The cells initially stored in liquid nitrogen were removed from the tank and quickly thawed in a water bath at 37°C. The vial contents were centrifuged, supernatant removed and the cells transferred into growth MEM medium supplemented with 10% Fetal Bovine Serum (FBS), 1% L-Glutamine and 1% antibiotic (Penicillin/Streptomycin) in a T75 culture flask and incubated at 37°C and 5% CO<sub>2</sub> to attain confluence.

### 2.4 Antiproliferative Assay

Upon attainment of confluence, cells were washed with saline phosphate buffer 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 100µl containing

2.0 ×10<sup>4</sup> cells/ml suspension was seeded in to a 96-well plate and incubated at 37°C for 24hrs at 5% CO<sub>2</sub> for 24hrs. After 24hrs, 15µl of sample extracts at seven different concentrations each serially diluted were added on Row H-B. Row A, containing media and cells alone served as the negative control. The standard drug Doxorubicin was used as the positive control. The experiment was done in triplicate. The cells were incubated for 48hrs, then 10 µl of MTT dye (5mg/ml) was added and the plates incubated for 2hrs at 37°C and 5% CO<sub>2</sub>. Formazan formation was confirmed using inverted light microscope and then solubilized with 50µl of 100% DMSO and optical density (OD) read using a calorimetric reader at 540nm and a reference wavelength of 720nm. The effect of the test samples on the cancer and normal cells was expressed as IC<sub>50</sub> values (the extracts concentration which kills 50% of the cancer cells) and CC<sub>50</sub> values (concentration of extracts that exerted cytotoxic effects to 50% of the normal cells) respectively [14]. Selectivity index (SI) which indicates the ability of the extracts to exert selective toxicity to cancer cells while sparing the normal ones was also calculated using:

$$SI = \frac{CC_{50}}{IC_{50}}$$

Where;

CC<sub>50</sub>– Concentration of extract that exerted cytotoxic effect to 50% of the normal cells

IC<sub>50</sub>– Concentration of extract that inhibited the growth of cancer cells by 50%

The data obtained was analysed using linear regression model to get IC<sub>50</sub> of each drug. The IC<sub>50</sub> values of the extracts were compared using Minitab Version 18 to obtain the Mean±SEM.

## 2.5 Gene Expression Assay

80% confluent (prostate) DU145 and (breast) HCC 1395 cancer cells, in T75 flasks, were treated with crude extracts of *A. gummifera*, *R. staddo* and *S. didymobotrya* extracts at concentrations equivalent to the calculated IC<sub>50</sub> values. Negative control cells were exposed to fresh growth media. After 48hrs, the media was decanted and cells washed in PBS to remove any debris. Trypsinization of the cells was done. RNA extraction was carried out using the procedure described by Pure Link RNA mini kit (Thermo Scientific, USA). The extracted RNA was quantified and its concentration and purity

examined using a Nanodrop ND-2000 spectrophotometer (Nanodrop Technologies, Inc., Wilmington, DE, USA).

Reverse transcription and cDNA amplification were done in single step reaction using SuperScript IV Reverse Transcriptase and Thermo scientific Real time SYBR green Kits according to the manufacturer's instructions. A single narrow peak from each PCR product was obtained by melting curve analysis at specific temperatures. The quantitative RT-PCR data was analyzed by a comparative threshold (Ct) method, and the fold inductions of the samples compared with the untreated samples. Glyceraldehyde 3-phosphate dehydrogenase was used as an endogenous control gene to normalize the expression of the target genes. The Ct cycle was used to determine the expression level in cells treated with different extracts after 48hours. Each sample was run in triplicate and the relative *p53* and *VEGF* mRNA expression calculated by 2-ΔΔCt

## 2.6 Qualitative Phytochemical Screening

Qualitative phytochemical screening of *S. didymobotrya*, *A. gummifera* and *R. staddo* was done using standard procedures as described by [15,16]. Secondary metabolites tested included alkaloids, saponins, phenols, flavonoids, glycosides, terpenoids and tannins.

### 2.6.1 Alkaloids

Three drops of Mayer's reagent were added to 2 ml of the extract. Formation of a yellow colored precipitate indicates the presence of alkaloids.

### 2.6.2 Saponins

Five milliliters of the extract were diluted with distilled water to 10 ml in a graduated cylinder and shaken for 10 minutes. Formation of a persistent layer of foam indicates the presence of saponins.

### 2.6.3 Phenols

Three to four drops of ferric chloride solution were added to the extract. Formation of a blue-black color indicates the presence of phenols.

### 2.6.4 Flavonoids

Two milliliters of dilute ammonia and 2 ml of concentrated sulphuric acid was added to the

extract. Formation of intense yellow color indicates the presence of flavonoids.

### 2.6.5 Glycosides

One milliliter glacial acetic acid was added to the 0.5 ml of the extract. One drop of iron chloride was added and the mixture shaken. 1 ml of concentrated sulphuric acid was then added to the mixture. Formation of a brown ring indicates the presence of glycosides

### 2.6.6 Terpenoids

Two milliliters of chloroform were added to 1 ml of the plant extract and shaken vigorously. 2 ml of concentrated sulphuric acid was then added and heated for 2 minutes. Formation of grey color indicates the presence of terpenoids.

### 2.6.7 Tannins

Five milliliters of distilled aqueous was added to 2 ml of the plant extract and heated to boil. 2 % of iron chloride was then added. A blue-black color formation indicates the presence of tannins.

## 3. RESULTS

### 3.1 Antiproliferative Assay

On the prostate cancer cell line (DU 145), the stem bark extract of *A. gummifera* MeOH: DCM exhibited the highest cell inhibition with an IC50 value of 3.34±0.05µg/ml followed by *R. staddo* root bark MeOH: DCM and *A. gummifera*

aqueous stem bark extracts at IC50 values of 9.36±0.10µg/ml and 18.29±0.02µg/ml, respectively (Table 1). Amongst all the *S. didymobotrya* extracts tested on the prostate cancer cell line, only the leaf MeOH: DCM extract portrayed activity with an IC50 value of 65.72±0.01µg/ml (Table 1).

On the breast cancer cell line (HCC 1396), *A. gummifera* root bark MeOH: DCM extract had the highest cell inhibition with an IC50 value of 2.38±0.01µg/ml. *A. gummifera* stem bark MeOH: DCM and *R. staddo* root bark MeOH: DCM extracts exhibited IC50 values of 6.07±0.04µg/ml and 15.71±0.04µg/ml respectively. *A. gummifera* stem bark and root bark aqueous extracts exhibited cell growth inhibition with IC50 values of 21.38±0.03µg/ml and 35.58±0.25µg/ml, respectively.

### 3.2 Cytotoxicity Assay

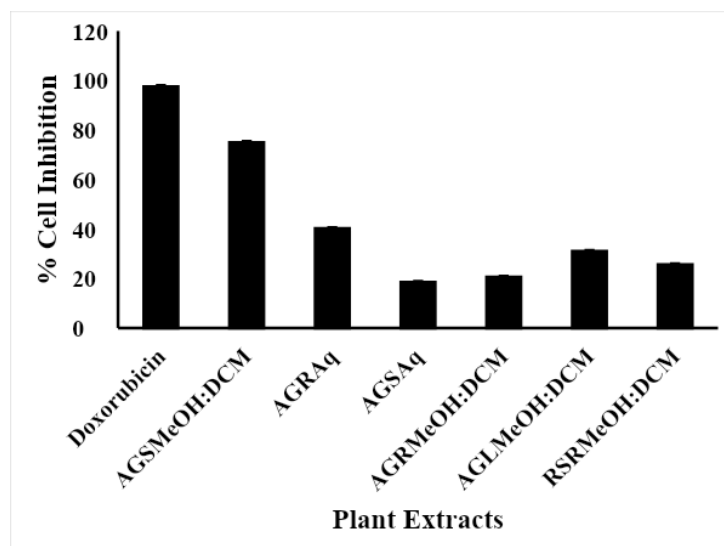
Treatment of VeroE6 cells with the standard drug doxorubicin (positive control) at a concentration of 100 µg/ml, resulted in inhibition of cell growth by 98.76% (CC50 = 0.98 ± 0.01 µg/ml). The stem bark MeOH: DCM extract of *A. gummifera* was the most cytotoxic among the plant extracts with a CC50 value of 15.68±0.08µg/ml, resulting in the inhibition of Vero cells by 75.05%. Treatment of Vero E6 cells with the aqueous and MeOH: DCM root, stem and leaf extracts of *A. gummifera*, and the *R. staddo* root bark MeOH: DCM extract led to the inhibition of cell survival by 40.23%, 18.5%, 20.55%, 30.98%, and 25.65% respectively (Fig. 1).

**Table 1. IC50 values of the plant extracts on the prostate and breast cancer cell lines**

Plant Sample	Part Used	Solvent	DU145 IC50 (µg/ml)	HCC 1395 IC50 (µg/ml)
<i>A. gummifera</i>	Stem bark	Aqueous	18.29±0.02f	21.38±0.03f
	Leaves	Aqueous	66.26±0.04b	>100
	Root bark	Aqueous	25.29±0.09e	35.58±0.25d
	Stem bark	MeOH: DCM	3.34±0.05h	6.07±0.04h
	Leaves	MeOH: DCM	64.48±0.24d	53.77±0.06c
<i>R. staddo</i>	Root bark	MeOH: DCM	79.71±0.10a	2.38±0.019i
	Root bark	MeOH: DCM	9.36±0.10g	15.71±0.04g
<i>S. didymobotrya</i>	Stem bark	Aqueous	>100	>100
	Leaves	Aqueous	>100	>100
	Root bark	Aqueous	>100	>100
	Stem bark	MeOH: DCM	>100	58.67±0.02b
	Leaves	MeOH: DCM	65.72±0.01c	32.32±0.03e
Doxorubicin	Root bark	MeOH: DCM	>100	65.06±0.07a
			0.24±0.03i	0.54±0.30j

Key: MeOH: DCM- methanol: dichloromethane

Values are expressed as Mean±SEM. Values that do not share a letter are significantly different (P≤0.05)



**Fig. 1. Percentage cell growth inhibition of the various plant extracts on normal VeroE6 cells**

Key; AGSMethOH:DCM- *A. gummifera* stem methanol: dichloromethane extract, AGRAq- *A. gummifera* root aqueous extract, AGSAq- *A. gummifera* stem aqueous extract, AGRMeOH:DCM- *A. gummifera* root methanol: dichloromethane extract, AGLMeOH:DCM- *A. gummifera* leaf methanol: dichloromethane extract, RSRMeOH:DCM- *R. staddo* root methanol: dichloromethane extract,

Doxorubicin was used as standard anticancer drug. Data is presented as Mean  $\pm$  SEM

### 3.3 Selectivity Index (SI) of, *A. gummifera*, *R. staddo* and *S. didymobotrya*

The greatest SI was observed on the root bark MeOH: DCM extract of *A. gummifera* on the breast cancer cell line (SI = 21.68). The aqueous stem bark and root bark; and stem bark MeOH: DCM extracts also showed a SI  $\geq$  3 in both prostate and breast cancer cells. A high selectivity (SI  $\geq$  3) was also observed on *R. staddo* root bark MeOH: DCM extract on the prostate and breast cancer cell lines tested (Table 2).

### 3.4 Assessment of Expression of Apoptotic (*p53*) and Angiogenic (*VEGF*) Genes

This study investigated the changes in *p53* and *VEGF* gene expressions in DU145 and HCC 1395 cancer cells. The relative mRNA expression levels of the genes were determined

using real time PCR. The fold increase or decrease in the expression of the genes was evaluated, relative to the calibrator (Relative Quantification=1). It was observed that all the extracts showed a significant fold increase in the *p53* expression compared to the control (Fig. 2). The MeOH: DCM extracts of *A. gummifera*, *R. staddo* and *S. didymobotrya* increased *p53* expression in DU145 by a fold change of 16.066, 15.985 and 14.99 and in HCC1396 by 11.99, 13.07 and 10.99 respectively.

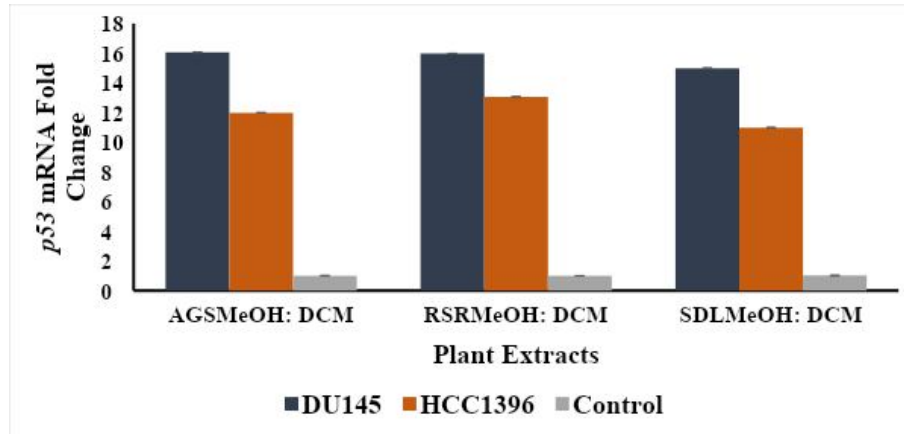
A downregulation of the *VEGF* gene was however noted on the cancer cells treated with *A. gummifera*, *R. staddo* and *S. didymobotrya* MeOH: DCM extracts with a fold change of 0.023, 0.042 and 0.035 in DU145 and by 0.031, 0.026 and 0.039 in HCC1396 respectively (Fig. 3). These extracts were selected due to the fact that they exhibited the most inhibition on the growth of the breast and prostate cancer cell lines.

**Table 2. Selectivity index of, *A. gummifera*, *R. staddo* and *S. didymobotrya* extracts**

Plant sample	Part used	Solvent	DU 145	HCC 1395
<i>A. gummifera</i>	Leaf	Aqueous	1.51	N/A
	Stem bark	Aqueous	3.44	3.94
	Root bark	Aqueous	3.28	3.33
	Leaf	MeOH: DCM	1.32	1.59
	Stem bark	MeOH: DCM	4.79	3.60
	Root bark	MeOH: DCM	0.65	21.68

<i>R. staddo</i>	Root bark	MeOH: DCM	5.15	3.03
<i>S. didymobotrya</i>	Leaf	MeOH: DCM	1.52	3.09
	Stem bark	MeOH: DCM	N/A	1.70
	Root bark	MeOH: DCM	N/A	1.53

Key: MeOH: DCM- Methanol: Dichloromethane; N/A-Not Applicable



**Fig. 2. Fold change in expression levels of *p53* gene on prostate and breast cancer cells by real time PCR**

Key; AGSMeOH:DCM- *A. gummiifera* stem methanol: dichloromethane extract, RSRMeOH:DCM- *R. staddo* root methanol: dichloromethane extract, SDLMeOH:DCM-*S. didymobotrya* leaf methanol: dichloromethane extract

Table 3. Phytochemical constituents of aqueous and methanol dichloromethane extracts of *S. didymobotrya*, *A. gummifera* and *R. staddo*

Plant	Part	Type of extract	Classes of phytochemicals						
			Alkaloids	Saponins	Phenols	Flavonoids	Glycosides	Terpenoids	Tannins
<i>S. didymobotrya</i>	Leaf	Aqueous	-	+	+	+	+	+	+
	Stem Bark	Aqueous	-	+	+	+	+	+	+
	Root Bark	Aqueous	-	+	+	+	+	+	+
	Leaf	MeOH: DCM	-	+	+	+	+	+	+
	Stem Bark	MeOH: DCM	-	+	+	+	+	+	+
	Root Bark	MeOH: DCM	+	+	+	+	+	+	+
<i>A. gummifera</i>	Leaf	Aqueous	-	+	+	+	+	+	+
	Stem Bark	Aqueous	+	+	-	+	+	+	+
	Root Bark	Aqueous	+	+	-	+	+	+	+
	Leaf	MeOH:DCM	+	+	+	+	+	+	+
	Stem Bark	MeOH: DCM	+	+	+	+	+	+	+
	Root Bark	MeOH: DCM	+	+	+	+	+	+	+
<i>R. staddo</i>	Root Bark	MeOH: DCM	+	+	+	+	+	+	+

(+) = Presence, (-) = Absence, MeOH: DCM- Methanol: Dichloromethane

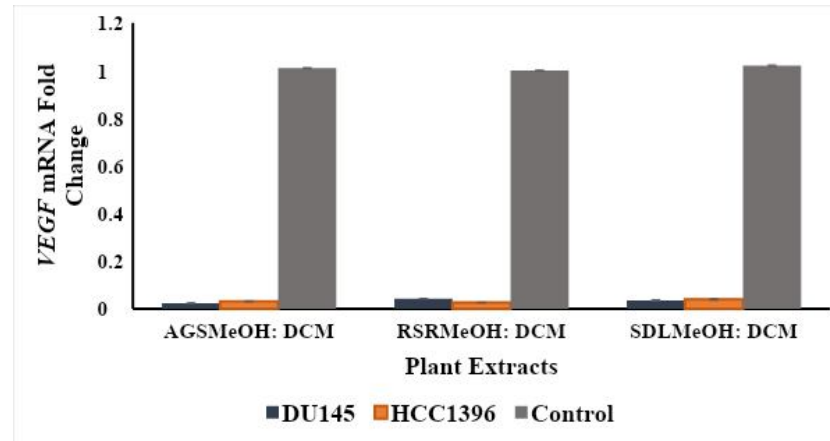


Fig. 3. Fold change in expression levels of *VEGF* gene on prostate and breast cancer cells by real time PCR

### 3.5 Qualitative Phytochemical Screening

Phytochemical screening demonstrated the presence of different types of phytochemicals including alkaloids, saponins, flavonoids, phenols, glycosides, tannins and terpenoids which could be responsible for the various pharmacological properties. Saponins, flavonoids, glycosides, terpenoids and tannins were found across all the plant extracts. Phenols were also found present in all extracts apart from the root and stem aqueous extracts of *A. gummifera*. Alkaloids were present in *A. gummifera* extracts except in the leaf aqueous extract. Alkaloids were also exhibited in *R. staddo* and *S. didymobotrya* roots methanol dichloromethane extracts (Table 3).

### 4. DISCUSSION

Generally, the plant extracts inhibited the proliferation of the cancer cells. The antiproliferative activities of the extracts were categorized based on median inhibitory concentration (IC50). The selective inhibitory activity of the extracts was determined and expressed as selectivity index (SI). The SI values demonstrate the differential in activity of the extracts on normal cells compared to cancerous cells. A high SI value depicts high selectivity. Medicinal plants with SI values of 2 or greater than 2 are considered to be highly selective. Selectivity index of less than 2 indicates less selectivity [17]. Both the aqueous and MeOH: DCM extracts of *A. gummifera* stem bark exhibited high growth inhibition on prostate and breast cancer cells. They also expressed a selectivity index greater than 3 indicating their selective toxicity to cancer cell lines while sparing the normal cells. *R. staddo* root bark MeOH: DCM extracts also showed antiproliferative effects on both the prostate and breast cancer cell lines with a selectivity index greater than 3. This demonstrates their potential as anticancer drugs.

p53 apoptotic gene controls various genetic expressions and plays an important role in cell proliferation and modulation of signal transduction pathways. In most cancer cases, the p53 gene is mutated, while in other cases it often possesses dysregulation of its upstream signaling pathways [18]. Evasion of apoptosis is considered to be one of the hallmarks of human cancers. Angiogenesis, on the other hand, is a physiological process of formation of new blood vessels on already existing ones. It has a vital role in supplying nutrients and oxygen and

excretion of metabolic waste. The newly formed blood vessels facilitate the metastatic dissemination of cancer cells. The lack of independent blood supply forces tumors to survive on the benefit of diffusion process which enable them to obtain oxygen and other nutrients from blood [19]. However, in the diffusion process tumors cannot grow beyond 2mm<sup>3</sup>. Progressively, absence of enough vasculature makes tumors to become hypoxic an event followed by Vascular Endothelial Growth Factor (VEGF) secretion which promotes neo angiogenesis towards the tumor and ultimately getting adequate blood supply to the cancer cells. In response to VEGF, blood vasculature starts growing towards tumor and provides nutrients to tumor [20]. In this study, the significant upregulation and downregulation of the P53 and VEGF genes respectively by the MeOH: DCM extracts of *A. gummifera*, *R. staddo* and *S. didymobotrya* is an indication that they inhibited the proliferation of prostate (DU145) and breast (HCC 1395) cancer cells via induction of apoptosis and by exhibiting anti-angiogenic effects. This study also investigated the pharmacologically important phytochemicals present in the plant extracts. Saponins, flavonoids, glycosides, terpenoids and tannins were found across all the plant extracts. A number of studies have been conducted to prove the protective effect of these phytochemicals against cancer [21]. A correlation has been observed between the phytochemicals and the plants antiproliferative activities against cancer cells [22]. These phytochemicals have been shown to possess antitumor properties [23]. They have also been shown to act as apoptotic and anti-angiogenic compounds [24]. The antiproliferative activities of these plants could be attributed to the phytochemicals present.

### 5. CONCLUSION

The plant extracts from *A. gummifera*, *R. staddo* and *S. didymobotrya* have been demonstrated to possess antiproliferative activity. However, both the aqueous and MeOH: DCM extracts of *A. gummifera* stem bark and the root bark MeOH: DCM extract of *R. staddo* exhibited the most promising and most selective cytotoxic activity. The mechanism of action of these antiproliferative activities can be linked to their upregulation of the p53 apoptotic gene and the downregulation of the angiogenic VEGF gene. The growth inhibitory potential of the plant extracts on the cancer cells and the probable mechanism of action could be attributed to the

presence of pharmacologically important phytochemicals. This study confirms that amidst the many traditional and pharmacological uses of these plants, they could also be used in the fight against cancer menace.

### ETHICAL APPROVAL

There were no humans involved in this study. The cell lines used were handled with a lot of care and professionalism and all protocols followed to the letter. All the safety standards in the place of study were observed and all measures considered to make sure that standard operating procedures were carried out maximally. Ethical approval was sought from Kenyatta University Graduate School Committee, Kenya Medical Research Institute (KEMRI), CTMDR Centre Scientific Committee (CSC) and Scientific and Ethics Review Unit (SERU) approval number KEMRI/SERU/CTMDR/O55/3535 before conducting the study.

### DISCLAIMER

This paper is an extended version of a preprint document of the same author.

The preprint document is available in this link: <https://www.biorxiv.org/content/10.1101/578948v1.full.pdf>

[As per journal policy, pre-print article can be published as a journal article, provided it is not published in any other journal].

### ACKNOWLEDGEMENTS

We are grateful to Kenya Medical Research Institute, Centre for Traditional Medicine and Drug Research fraternity for their unending support, guidance and co-operation in actualizing this work. We acknowledge the KEMRI's Internal Research Grant (IRG) for funding Dr. Peter Mwitari through SSC 2902 who supplied us with laboratory consumables and the cancer cell lines. We are also thankful to the Pyrethrum project funding, SSC 2969 that facilitated the purchase of the q-PCR and CO<sub>2</sub> incubator, equipment's that aided in the success of this work.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. *International Journal of Cancer*. 2021;149(4):778-89.
2. Korir A, Okerosi N, Ronoh V, Mutuma G, Parkin M. Incidence of cancer in Nairobi, Kenya (2004–2008). *International Journal of Cancer*. 2015;137(9):2053-9.
3. Aruna MS, Prabha MS, Priya NS, Nadendla R. *Catharanthus roseus*: ornamental plant is now medicinal boutique. *Journal of Drug Delivery and Therapeutics*. 2015;1-4.
4. Agbor AM, Naidoo S. Plants used by african traditional healers in the management of oral diseases: A review. *International Journal of Research-Granthaalayah*. 2019;7(8):273-86.
5. Raj A, Vinnarasi J, Venkataraman R, Augustin M. HPTLC fingerprinting analysis of tannin profile on canthium coromandelicum and flueggea leucopyrus willd. *Research Journal of Pharmacy and Technology*. 2018;11(12):5355-8.
6. Umamaheswari A, Prabu SL, John SA, Puratchikody A. Green synthesis of zinc oxide nanoparticles using leaf extracts of *Raphanus sativus* var. *Longipinnatus* and evaluation of their anticancer property in A549 cell lines. *Biotechnology Reports*. 2021;29:e00595.
7. Pandey P, Mehta R, Upadhyay R. Physico-chemical and preliminary phytochemical screening of *Psoralea corylifolia*. *Archives of Applied Science Research*. 2013; 5(2):261-5.
8. Breidenbach M, Rein DT, Schöndorf T, Khan KN, Herrmann I, Schmidt T, Reynolds PN, Vlodaysky I, Haviv YS, Curiel DT. A new targeting approach for breast cancer gene therapy using the heparanase promoter. *Cancer Letters*. 2006;240(1):114-22.
9. Samuel TA, James AB, Oshodi TA, Odii UO, Chidume I, Makinde OE, Oguntoye LA, Magbagbeola OA. Evaluation of retinoblastoma (Rb) and protein-53 (p53) gene expression levels in breast cancer cell lines (MCF-7) induced with some selected cytotoxic plants. *Journal of Pharmacognosy and Phytotherapy*. 2013;5(7):120-6.
10. Bieging KT, Mello SS, Attardi LD. Unravelling mechanisms of p53-mediated tumour suppression. *Nature Reviews Cancer*. 2014;14(5):359-70.

11. Sagar SM, Yance D, Wong RK. Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer—Part 1. *Current Oncology*. 2006;13(1):14-26.
12. Izawa JI, Dinney CP. The role of angiogenesis in prostate and other urologic cancers: A review. *Cmaj*. 2001;164(5):662-70.
13. Xiao D, Singh SV. Z-Guggulsterone, a constituent of Ayurvedic medicinal plant *Commiphora mukul*, inhibits angiogenesis *in vitro* and *in vivo*. *Molecular Cancer Therapeutics*. 2008;7(1):171-80.
14. Nemati F, Dehpouri AA, Eslami B, Mahdavi V, Mirzanejad S. Cytotoxic properties of some medicinal plant extracts from Mazandaran, Iran. *Iranian Red Crescent Medical Journal*. 2013;15(11).
15. Ochwang IDO, Kimwele CN, Oduma JA, Gathumbi PK, Kiama SG, Efferth T. Phytochemical screening of medicinal plants of the Kakamega Country, Kenya commonly used against cancer. *Med. Aromat. Plants*. 2016;5(6).
16. Anthony Swamy T, Ngule MC, Jackie K, Edwin A, Ngule ME. Evaluation of *in vitro* antibacterial activity in *Senna didymobotrya* roots methanolic-aqua extract and the selected fractions against selected pathogenic microorganisms. *International Journal of Current Microbiology and Applied Sciences*. 2014; 3(5):362-76.
17. Badisa RB, Darling-Reed SF, Joseph P, Cooperwood JS, Latinwo LM, Goodman CB. Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF-7 cells. *Anticancer Research*. 2009;29(8):2993-6.
18. Brown AK, Papaemmanouil A, Bhowruth V, Bhatt A, Dover LG, Besra GS. Flavonoid inhibitors as novel antimycobacterial agents targeting Rv0636, a putative dehydratase enzyme involved in *Mycobacterium tuberculosis* fatty acid synthase II. *Microbiology*. 2007;153(10):3314-22.
19. Kumar MA, Ojha NK, Kumar A. Prospective role of Indian medicinal plants in inhibiting vascular endothelial growth factor (VEGF) mediated pathological angiogenesis. *J Homeop Ayurv Med*. 2013;2(121):2-5.
20. Missiaen R, Mazzone M, Bergers G. The reciprocal function and regulation of tumor vessels and immune cells offers new therapeutic opportunities in cancer. In *Seminars in Cancer Biology*. 2018;52:107-116. Academic Press.
21. Chen G, Li X, Saleri F, Guo M. Analysis of flavonoids in *Rhamnus davurica* and its antiproliferative activities. *Molecules*. 2016;21(10):1275.
22. Jin-Jun HO, Yao SH, Zhou YA, Lin FA, Lu-Ying CA, Shuai YA, Hua-Li LO, Wan-Ying WU, De-An GU. Anti-proliferation activity of terpenoids isolated from *Euphorbia kansui* in human cancer cells and their structure-activity relationship. *Chinese Journal of Natural Medicines*. 2017;15(10):766-74.
23. Martino E, Casamassima G, Castiglione S, Cellupica E, Pantalone S, Papagni F, Rui M, Siciliano AM, Collina S. Vinca alkaloids and analogues as anti-cancer agents: Looking back, peering ahead. *Bioorganic and Medicinal Chemistry Letters*. 2018;28(17):2816-26.
24. Ren Y, Wang C, Xu J, Wang S. Cafestol and kahweol: a review on their bioactivities and pharmacological properties. *International Journal of Molecular Sciences*. 2019;20(17):4238.

---

© 2022 Mbugua et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.