EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN: 2663-0990 (Print) & ISSN: 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

Volume-5 | Issue-1 | Jan-Feb- 2023 | DOI: 10.36349/easjpp.2023.v05i01.002

OPEN ACCESS

Original Research Article

In vitro Cytotoxic Activities of *Catha edulis* (Vahl) Forssk. Ex Endl. (Khat) Varieties from Kenya on Select Cancer Cell Lines

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Article History
Received: 21.12.2022
Accepted: 25.01.2023
Published: 04.02.2023

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: Khat (*Catha edulis* (Vahl) Forssk.) is a herb from the *Celastraceae* family (also known as *qat*, *gaad*, or *miraa*). The leaves and stems are used medicinally and for recreational purposes. The communities that grow khat have identified different varieties based on perceived appearance and quality. This study aimed to evaluate the cytotoxicity of khat varieties grown in the Meru and Embu Counties of Kenya. Field studies were undertaken in the markets and farms in Meru and Embu Counties of Kenya to document and purchase local khat varieties. Dried khat was extracted with a 1:1 v/v MeOH: CH_2C_{l2} solvent and water. Cytotoxicity of extracts was determined *in vitro* by MTT assay against four normal and cancer cells namely; HeLa ATCC® CCL-2™, HCC1395 ATCC® CRL-2324™, Hep2 ATCC® CCL-23™, and Vero E6 ATCC® CRL-1586™. The khat varieties identified were *Muti Mutiiri*, *Mugwanthingi*, *Gicheru*, *Karimi ka Nthiya*, *Muguka*, *Black colombo asili*, *Black mbaine*, and *white*. The aqueous extracts of *black colombo asili* and *black mbaine displayed the* highest cytotoxic activity against HeLa cell lines having IC_{50} 37.15 ± 1.75 µg/ml and 38.31 ± 2.05 µg/ml, respectively. *Muguka* and *Muti Mutiiri* varieties were not cytotoxic to the Vero E6 cell line with CC_{50} > 100 µg/ml. 75% (12/16) of extracts were cytotoxic to the Vero E6 cell line with CC_{50} < 100 µg/ml. This study demonstrated that there is variability in activities between the identified khat varieties. Toxicity was observed *in vitro* due to observed cytotoxicity to the Vero E6 cell line.

Keywords: *Catha edulis*, Khat, Cytotoxicity, Anticancer.

INTRODUCTION

Catha edulis is a plant whose young leaves and stems are chewed by an estimated 20 million people globally as a psychostimulant (Bogale *et al*., 2016). It is a flowering evergreen plant indigenous to much of Africa and cultivated as a tree or shrub throughout East Africa and the Arabian Peninsula (Baariu & Mulaku, 2015; Carrier, 2006). Various countries and communities have different names for the plant, such as Chat and Qat in Yemen and Ethiopia, Qaad and Jaad in Somalia, Miraa and Muguka in Kenya, Jimma in the Oromo language, and bushman's tea (South Africa). It is known as khat in most western countries (Luqman & Danowski, 1976; Ngari *et al*., 2018).

Cathinone, cathine and norephedrine are primarily responsible for the stimulating properties of khat (Ketema *et al*., 2015). Cathinone is an intermediate metabolite in the biosynthesis of cathine which is found mainly in young fresh leaves of the khat plant (Kalix, 1992). Cathinone is an unstable compound that decomposes within a few days of being picked and transforms into cathine and norephedrine if the leaf is dried (Al-Motarreb *et al*., 2002). Other phytochemicals found in khat include merucathinone, pseudomerucathine, and merucathine (Al-Motarreb *et al*., 2002) .

Different communities that cultivate Khat classify the various varieties based on perceived quality and appearance (Makandi Mworia Bsc MLS *et al*., 2017). Farmers in Yemen distinguish four varieties based on the color of the shoots and developing twigs: '*Abyadh*,' (pale green), *Azraq* (purplish), *Aswad* (crimson), and *Ahmar* (red), which is intermediate between *Azraq* and *Aswad (*Al-Motarreb *et al.,* 2002*;* Krikorian*,* 1984*)*. In Ethiopia,

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the two significant cultivars are *Dimma* (red) or *Ahde* (white). Getahun and Krikorian classified khat into three types, 'madness-causing, intoxicating-like spirit, and insomnia-causing based on their effects' (Ayana & Mekonen, 2004; Krikorian & Getahun, 1973). There are forty kinds of khat in Yemen (Al-Habori *et al*., 2002), whereas in Kenya, up to 30 traditional varieties have been identified from Embu and Meru counties. Some common varieties are *Kiira gikiiru (Asili), Kigwe* and *muguka (*Kiunga *et al.,* 2016*;* Ngari *et al.,* 2018*).*

Apart from its usage as a social and recreational stimulant, khat leaves, stems, and roots are used to treat various illnesses, including influenza, cough, asthma, malaria, gonorrhoea, vomiting, headaches, and infertility in men (Al-Hebshi & Skaug, 2005; Balint *et al*., 2009; Ketema *et al*., 2015; Kiunga *et al*., 2016; van Wyk & Prinsloo, 2019) . Previous studies reported cytotoxicity of khat extracts on rat pancreatic β- cells (RIN- 14B) (Alsalahi *et al*., 2018), peripheral blood mononuclear cells (PBMCs) (Murdoch *et al*., 2011), leukemia cells (Bredholt *et al*., 2009; Dimba *et al*., 2003), and breast cancer cells (Lu *et al*., 2017). These studies suggest that khat can be explored in the search for novel drug candidates. Cytotoxicity of the *Asili* variety of Kenyan khat has been reported (Bredholt *et al*., 2009). However, there are no reports on the cytotoxicity profile of other khat varieties cultivated in Kenya. Therefore, the objective of this study was to evaluate the *in vitro* cytotoxic effects of extracts of Kenyan khat varieties.

MATERIALS AND METHODS

Collection of plant materials

The area of study was Meru and Embu counties of Kenya. Meru County is to the eastern region of Kenya, approximately 225 kms north east of the capital Nairobi. It covers a geographical area of 6, 936 km^2(*Meru County - Kenya National Bureau of Statistics*, n.d.). Embu county is located 120 kms northeast of Nairobi, covering a geographical area of 2, 818 km^2(*Embu County - Kenya National Bureau of Statistics*, n.d.) Bundles of shoots and small branches of khat from the markets and farms in Meru and Embu counties were purchased. The local names of the different khat varieties were noted. The Plants were identified by a taxonomist. They were assigned voucher specimen numbers and deposited at the University of Eldoret Herbarium. The khat bundles were dried in the shade for one week, and the resultant dry material was pulverized (Christy and Noris Ltd) to powder and stored.

Preparation of plant extracts

The powdered plant material was weighed (300g) and placed in an Erlenmeyer flask. The plant materials were extracted with Methanol and Dichloromethane in a 1:1 v/v., and left to stand for 48 hours, then decanted and filtered through a Whatman No. 1 filter paper. The filtrate was subjected to reduced pressure using a Buchi rotary evaporator, and extracts stored at 4° C until further use.

Aqueous extracts of khat plants were prepared by placing 100g of plant material in an Erlenmeyer flask. Distilled water was added to cover, and the flask was placed in a water bath set at 60°C for 1 hour. The extract was decanted and filtered with Whatman No. 1 filter paper. The filtrate was lyophilized using a freeze dryer (Modulyo). The dried extracts were stored in a desiccator.

Cell culture

Cells were obtained from American Type Culture Collections (ATCC). The cell lines used for this study were HeLa (ATCC® CCL-2™), HCC1395 (ATCC® CRL-2324™), Hep2 (ATCC® CCL-23™) and Vero E6 (ATTC® CRL-1586™)). The cells were cultured in Eagles' Minimum Essential Medium (EMEM, Sigma) containing 10% heat inactivated Foetal Bovine Serum (FBS, Sigma), 1% antibiotic (100U/ml penicillin, 100µg/ml streptomycin Sigma), 37°C, 5% CO_2 with 95% relative humidity.

Cytotoxicity assay

The cytotoxic activity of sixteen extracts from eight varieties of khat were assessed by the MTT assay (MTT is the abbreviation for 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Irungu *et al*., 2014). Briefly, 2 x 10^4 cells/well suspensions were seeded in 96- well microtiter plates and incubated at 37 °C / 5 % CO_2 for 24hrs to allow for attachment. Khat extracts were added to attached cells over a concentration range of 1000 µg/ml to 1.4 µg/ml. Untreated wells with cells served as controls, while wells with media without cells served as blanks. Plates were incubated for 48 hours at 37 °C / 5 % CO_2, thereafter, 10 µL of MTT dye (5 mg/ml) was added and further incubated for 4 hours. Media was aspirated off the wells and 40 µl DMSO added to each well. The absorbance was read on an ELISA spectrophotometer (Multiscan Go), at 570nm and 620nm. The standard drug doxorubicin was used as a reference drug. The assays were carried out in triplicate. The results were obtained as optical density, transferred into the Microsoft Excel and compared to the untreated controls. The extract concentration required for 50% (IC_{50}) inhibition of cells was calculated using an inherent program in GraphPad Prism software.

Cytotoxic activity of extracts against cancer cells was classified as IC_{50} of < 10 µg/ml strong activity, >10 - 50 µg/ml moderate activity, >50 - 100µg/ml low activity and > 100 µg/ml inactive (Indrayanto *et al*., 2021). Extracts with cytotoxic activity on normal Vero cells (CC_{50}) of ≤100 µg/ml were considered strongly cytotoxic (Indrayanto *et al*., 2021)

Data analysis

Extract concentration causing 50% inhibition (IC_{50}) or cytotoxic concentrations (CC_{50}) compared to the control cell growth (100%) of each was calculated using nonlinear regression analysis in GraphPad Prism 8 software. Results were reported as mean ± SEM. Statistical significance (p-value < 0.05) was established through comparison of the mean IC_{50} values of extracts and the standard reference drug using One way ANOVA, with Dunnett's multiple comparison tests.

Ethical considerations

The study was ethically approved by Kenya Medical Research Institute, Scientific Steering Committee (SSC 2949).

RESULTS AND DISCUSSION

This study collected eight varieties of khat from the Meru and Embu counties of Kenya, as shown in Table 1. Khat varieties collected from Embu County were *Muti Mutiiri*, *Mugwanthingi*, *Gicheru*, *Karimi ka Nthiya*, and *Muguka*, whereas *black colombo asili*, *black mbaine*, and *white* were collected from Meru County in Kenya. Previous studies have documented the traditional varieties of *Mugwanthingi* (Kiunga *et al*., 2016), *Muti Mutiiri*, and *Muguka* (Kiunga *et al*., 2016; Ngari *et al*., 2018). The black varieties of khat collected in this study have previously been reported as *miraa miiru (*Carrier, 2006*)* and *gikiiru/asili/nyeusi* and are thought to be the preferred variety among khat chewers (Kiunga *et al*., 2016). Old khat trees are referred to as *mbaine* among the Ameru people and are thought to date back over three centuries; thus, the *black mbaine* variety was harvested from an old khat tree (Carrier, 2006; Kiunga *et al*., 2016). The white variety was previously recorded as *miraamieru* ('white miraa') in Kenya (Kiunga *et al*., 2016) and *ahde* in Ethiopia (Krikorian & Getahun, 1973). There is no previous record of *gicheru* and *Karimi ka Nthiya* in literature. This may be as a result of diverse dialects or phonetics existing within communities that reside in Meru and Embu counties (Kiunga *et al*., 2016). An inquiry needs to be undertaken to determine if the two are new varieties or if there exists a similarity between *gicheru* and *gikieru*; and whether *Karimi ka Nthiya* is similar to *mugukawakarimi* as previously reported (Kiunga *et al*., 2016; Ngari *et al*., 2018).

Table 1: *Catha edulis* varieties collected in Meru and Embu Counties of Kenya

Variety	Part	County	Latitude	Longitude	Voucher No.
Muti Mutiiri	Leaves and Shoots	Embu	S0.655054	E37.475845	KM2017/020
Mugwanthingi	Leaves and Shoots	Embu	S0.45341356	E37.48607002	KM2017/008
Gicheru	Leaves and Shoots	Embu	S0.655054	E37.475845	KM2017/022
Karimi ka Nthiya	Leaves and Shoots	Embu	S0.655054	E37.475845	KM2017/019
Muguka	Leaves and Shoots	Embu	S0.45341356	E37.48607002	KM2017/007
Black Colombo Asili	Leaves and Shoots	Meru	N0.363307	E37.938435	KM2017/029
Black (Mbaine)	Leaves and Shoots	Meru	N0.366564	E37.937904	KM2017/032
White	Leaves and Shoots	Meru	N0.366564	E37.937904	KM2017/033

The water and MEOH: CH_2Cl_2 extracts of eight Kenyan varieties of khat were tested for cytotoxicity against human cervical cancer (HeLa), human HeLa derived cells (Hep2), breast cancer (HCC 1395), and a normal cell line derived from African green monkey kidney (Vero E6) cell line as shown in **Table 2**. To the best of our knowledge, this is the first report that compared the cytotoxic properties of khat varieties found in Kenya. The aqueous extract of *black colombo asili* had the highest activity against HeLa cell lines with IC_{50} of 37.15 ±1.75 All extracts of *Muguka* and *Muti Mutiiri*, varieties were not cytotoxic to the non-cancerous Vero E6 cell line with CC_{50} > 100 µg/ml (Indrayanto *et al*., 2021).

Table 2: Cytotoxic activity of *Catha edulis* varieties from Meru and Embu Counties in Kenya

Khat variety	Extract	IC_{50} (µg/ml) ± SEM			CC_{50} (µg/ml)
		HCC 1395	HeLa	Hep2	Vero
Muti Mutiiri	MeOH: CH_2Cl_2	46.32 ±3.98^{c}	103.8±3.75^{d}	54.58±4.13^{d}	156.9± 0.35^{d}
	H_2O	66.68±1.23^{d}	71.72±1.62^{d}	70.68±4.66^{d}	134.5±4.0^{d}
Mugwanthingi	MeOH: CH_2Cl_2	68.79±1.27^{d}	105.0±2.65^{d}	54.03±4.15^{d}	144.5±2.15^{d}
	H_2O	74.93±2.58^{d}	98.66±0.49^{d}	119.2±4.4^{d}	77.93±11.36^{d}
Gicheru	MeOH: CH_2Cl_2	127.1±3.45^{d}	119.3±5.35^{d}	45.4±6.53^{d}	72.34±2.87^{c}
	H_2O	87.63±2.17^{d}	88.27±2.23^{d}	131.1±7.45^{d}	152.0±7.5^{d}
Karimi ka Nthiya	MeOH: CH_2Cl_2	192.6±4.05^{d}	107.6±2.6^{d}	63.86±7.41^{d}	68.65±3.8^{c}
	H_2O	275.8±8.45^{d}	111.8±1.6^{d}	78.1±1.37^{d}	52.89±2.49^{b}
Muguka	MeOH: CH_2Cl_2	82.95±11.90^{d}	118.5±0.7^{d}	70.89±2.68^{d}	248.7±2.15^{d}
	H_2O	136.5±2.0^{d}	373.7±4.7^{d}	429.9±10.8^{d}	544.5±2.7^{d}

Black Colombo Asili	MeOH: CH_2Cl_2	159.1±2.6[d]	119.0±1.2[d]	44.03±0.68[c]	271.6±4.25[d]
	H_2O	90.03 ±3.48[d]	37.15 ±1.75[d]	85.24±6.87[d]	45.73±7.92[b]
Black Mbaine	MeOH: CH_2Cl_2	259±8.3[d]	101.6±1.35[d]	76.75±10.68[d]	193.3±3.35[d]
	H_2O	110.6±3[d]	38.31 ±2.05[d]	105.4±4.85[d]	19.24±1.09
White	MeOH: CH_2Cl_2	108.6±3.15[d]	105.9±3.55[d]	67.48±2.13[d]	140.8±1.5[d]
	H_2O	121.6±2.05[d]	107.1±0.55[d]	101.5±0.8[d]	98.65±0.92[d]
Doxorubicin		0.13±0.08	2.06±0.39	0.77±0.32	5.32±0.41

[a]P < 0.05, [b]P < 0.01, [c]P < 0.001, [d]P < 0.0001

A selectivity index value of ≥ 2 is considered promising as it indicates that a drug candidate is twice more cytotoxic to a cancer cell compared to a normal cell line (de Oliveira *et al*., 2016; Koch *et al*., 2005). In this study, the MeOH: CH_2Cl_2 of *black colombo asili* was highly selective to Hep2 cell lines (SI- 6.1) compared to the normal Vero cells as shown in **Table 3**. Generally, the khat extracts were more selective to Hep 2 and HCC 1395 cells at 31.25% (5/16) each, compared to HeLa cells at 12.5 % (2/16).

Table 3: Selectivity Index of khat extracts

Khat variety	**Extract**	**Selectivity Index**		
		HCC 1395	**HeLa**	**Hep2**
Muti Mutiiri	MeOH: CH_2Cl_2	3.39	1.51	2.87
	H_2O	2.02	1.89	1.90
Mugwanthingi	MeOH: CH_2Cl_2	2.1	1.38	2.67
	H_2O	1.04	0.79	0.65
Gicheru	MeOH: CH_2Cl_2	0.57	0.61	1.59
	H_2O	1.73	1.72	1.16
Karimi ka Nthiya	MeOH: CH_2Cl_2	0.36	0.64	1.08
	H_2O	0.19	0.47	0.68
Muguka	MeOH: CH_2Cl_2	3.0	2.10	3.51
	H_2O	3.99	1.46	1.27
Black Colombo Asili	MeOH: CH_2Cl_2	1.71	2.28	6.1
	H_2O	0.51	1.23	0.54
Black Mbaine	MeOH: CH_2Cl_2	0.75	1.90	2.52
	H_2O	0.17	0.5	0.18
White	MeOH: CH_2Cl_2	1.30	1.33	2.09
	H_2O	0.81	0.92	0.97
Doxorubicin		40.92	2.59	6.91

The aqueous extracts of *black colombo asili* and *black mbaine* displayed the highest cytotoxic activity against HeLa cell lines having IC_{50} 37.15 ± 1.75 µg/ml and 38.31 ± 2.05 µg/ml respectively. Furthermore, other khat extracts with IC_{50} of < 50 µg/ml include MeOH: CH_2Cl_2 of *Black colombo asili* and *Gicheru* against Hep2 cells and MeOH: CH_2Cl_2 of *Muti Mutiiri* against HCC 1395 cells. Previous studies report the anticancer activity of khat *asili* variety against leukemia cell lines *in vitro* (Bredholt *et al*., 2009; Dimba *et al*., 2003). The morphological traits of traditional black varieties are crimson red twigs (Kiunga *et al.*, 2016) and are comparable to the *Aswad* variety in Yemen and *dimma* in Ethiopia (Al-Motarreb *et al*., 2002).The black variety of khat is reported to be preferred by locals for consumption as it is thought to be easy to chew and have fewer side effects, such as insomnia (Kiunga *et al*., 2016).

The khat varieties inhibited the growth of cancer cells at IC_{50} 37.15 - 98.66 µg/ml. This variability in activity can be attributed to the fact that chemical constituents of khat are dependent on the origin, environment/location, method of cultivation, age of the plant, and time of harvest (Al-Motarreb *et al*., 2002; Geisshüsler & Brenneisen, 1987). A comparison of khat from Kenya, Madagascar, and Ethiopia showed that the highest level of cathinone occurred in khat bundles originating from trees and sold in Nairobi's Street market (Geisshüsler & Brenneisen, 1987)

The extracts of *Mugwanthingi*, *Gicheru*, *Karimi ka Nthiya*, *black colombo asili*, and *black mbaine* displayed high to moderate cytotoxicity to the normal Vero cell line. A similar trend was observed in khat varieties from Saudi Arabia where cytotoxicity of khat extracts was more cytotoxic to normal human fetal lung fibroblast cells (MRC5) as compared to human breast cancer (MCF-7), human colon cancer (HT-29), and human ovary cancer (A2780) cell lines (Alsanosy *et al*., 2020). A study by Lu *et al.*, (Lu *et al*., 2017) on the effects of khat on the breast cancer cell line MDA -MB-231 demonstrated that khat extracts induced apoptosis at 400 µg/ml. However, the study did not use a normal cell line for comparison.

It is important to follow up *in vitro* pre-clinical anticancer screening with *in vivo* studies. The *in vitro* studies are limited in that they cannot mimic *in vivo* conditions fully, resulting in activity observed *in vitro* not translating to activity *in vivo* and vice versa (López-Lázaro, 2015). Furthermore, the study was carried out using dried plant material. Khat is normally consumed while still fresh for a stimulating effect.

CONCLUSION

The study evaluated sixteen extracts of eight traditional varieties of khat found in Kenya for cytotoxicity. The MEOH: CH_2Cl_2 extracts of *Muti Mutiiri* and *black colombo asili* inhibited HCC 1395 and Hep2 respectively selectively (SI >2) compared to the normal Vero cells. Twelve extracts of *Mugwanthingi*, *Gicheru*, *Karimi ka Nthiya*, *black colombo asili* and *black mbaine* displayed high to moderate cytotoxicity to the normal Vero cell line. The cytotoxicity observed may limit use as an ethnomedicine.

ACKNOWLEDGEMENTS

This work was supported through a grant by Kenya Medical Research Institute. We want to thank the County government of Meru and Embu agricultural officers and the farmers for the valuable information accorded to the group. We also want to thank Ms. Margaret Wanjohi of CTMDR, KEMRI, for assisting in sample extraction

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Cite This Article: Lilian C. Ngeny, Lucia K. Keter, Beatrice N. Irungu, James M. Kuria, Nicholas M. Mwikwabe, Joyce M. Ondicho, Richard K. Korir, Peter G. Mwitari, Festus M. Tolo, Jennifer A. Orwa (2023). *In vitro* Cytotoxic Activities of *Catha edulis* (Vahl) Forssk. Ex Endl. (Khat) Varieties from Kenya on Select Cancer Cell Lines. *EAS J Pharm Pharmacol, 5*(1), 8-13