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Anti-Cancer Activities of Crude Extracts from Kenyan Moringa Oleifera Lam and Rauwolfia Caffra against Selected Cancer Cell Lines

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Abstract: *The current study was undertaken to validate the traditional use and determine the safety profiles of Moringa oleifera and Rauwolfia caffra used in Kenya to manage tumors and related ailments. This was achieved by determining the anti-proliferative activities of the active antioxidant extracts (50% methanol in dichloromethane) of Moringa oleifera leaves (MeOH) and Rauwolfia caffra stem bark using crystal violet assay. Human liver (hepatocellular carcinoma, Hep-G2) and muscular (rhabdomyosarcoma, RD) were used as model cell lines and cytotoxicity was assessed using Vero cell lines. The results of this work showed that the methanolic extract of the leaves of Moringa oleifera displayed significant anti-proliferative activity ($p < 0.05$) against Hep-G2 and RD cell lines with limited activity on normal Vero cells. Comparatively, RD cell lines were more sensitive than Hep-G2. The extract of the stem bark of Rauwolfia caffra did not show significant activity against proliferation of RD and Hep-G2 cells, however, it exhibited high activity against the proliferation of Vero cells. From this studies Moringa oleifera was found to be less toxic and to possess anticancer activities, while R. caffra displayed modest anticancer activities and high toxicity levels against normal Vero cells. The efficacy and safety profiles as observed in this study provides a validated evidence of anticancer activity of the two plants that should be investigated further for rational therapeutic designs.*

Keywords: Anticancer activities, Hep-G2 cell lines, *Moringa oleifera, Rauwolfia caffra*, RD cell lines

I. Introduction

Cancer cases have been on the rise in recent years leading to increased morbidity and mortality; accounting for approximately 63% deaths in developing countries in 2008 [1]. All forms of cancer are incurable and current drugs have many side effects prompting the urgent need to search for the next generation therapy. Complementary and alternative sources of anticancer drugs have been exemplified by pacliaxel (Taxus brevifolia) and vinca alkaloids (periwinkle plant, Catharanthus roseus). These are plants used traditionally to manage or treat cancer and related diseases [2]. Kenya has a rich biodiversity and rich folklore on use of medicinal plant for the treatment of various ailments including cancer [3-5]. Close to 80 % of Kenya's population especially in the rural areas use traditional medicine for primary health care. This is partly due to low coverage of conventional primary health care facilities and other socio-economic factors such as cost of conventional medicines and flexible modes of payment for services of traditional practitioners. The medicinal plants investigated in the present study, Moringa oleifera and Rauwolfia caffra have been previously documented to be used traditionally to treat/manage cancer and related diseases among other diseases [3-5].

Moringa oleifera Lam. also known as the horseradish, drumstick tree or Ben oil tree in English and traditionally as Mlongo in Kiswahili belongs to the monogeneric family Moringaceae, [6-7]. This plant is endemic to Northwest India, Pakistan, Bangladesh and Afghanistan but has been naturalized in the low attitudes of coastal regions of East Africa. [3-4, 6-7]. Traditionally, different parts of this plant are used as remedy for a number of ailments [3-4, 7-9]. In Western Kenya where ethnobotanical survey of anticancer plants was undertaken, the seed oil also known as Ben oil, is used in poultices to relieve painful body swellings and other related skin infections [3-4]. The main phytochemical feature of the leaves of Moringa oleifera include; quercetin-3-O-glucoside and Kaempferol-3-O-glucoside which have good antioxidant activities as they scavenge free radicals thus reducing oxidative stress [10]. Various studied have suggested that the anticancer and chemopreventive property of Moringa oleifera could be attributed to its constituent compound called niazimicin [11-12]. The present study also investigated the anti-cancer potential of Rauvolfia caffra Sond. also

known as the quinine tree in English and locally as Kumunandebe by Luos and Omumure by the Abakuria people in Western Kenya. The plant belongs to the genus of evergreen trees and shrubs in the dogbane family, Apocynaceae [13]. The English name quinine refers to the bitter and supposedly quinine-like properties of the bark [3]. This plant is widely distributed in Africa; in Kenya, Tanzania and Southern Africa, it is found in riverine Brachystegia woodland, lowland forests, dry and wet montane forests [3]. Quinine tree is used as a medicinal plant among traditional communities in many African countries; in Western Kenya the bark decoction is drunk as a medicine for general body swellings, rheumatism and pneumonia [4,6,14].

Previous studies have shown that the ethanolic extract of the roots of this plant have exhibited good antimycobacterial and antioxidant activities [15]. The phytochemistry of the stem bark and the roots of Rauvolfia caffra consists of mainly indole alkaloids; reserpine, which is used to treat hypertension [16-18]. It is possible that the alkaloids play an important role in the medicinal properties of this plant [19]. These compounds have strong conjugation systems associated with high antioxidant potential and therefore capable of managing tumors and other degenerative diseases caused by reactive oxygen species (ROS) [20].

Prior to the anti-proliferative test, the antioxidant potential of different extracts of the two plants, obtained from varied solvent systems was determined. These studies revealed that the MeOH extract of the leaves of M. oleifera and the stem bark extract (50 % MeOH in CH_2Cl_2) of R. caffra showed the highest radical scavenging activity and therefore were tested for their antiproliferative potential. These antioxidant results are consistent with those reported by Lalas and Tsaknis, 2002 and Siddhuraju and Becker, 2003 [21-22].

Previous related studies that evaluated the anticancer potential of M. oleifera targeted mostly the aqueous extract (the commonly used solvent for extraction, in traditional medicine) which in some instances is not the most bioactive extract [23-24]. The aqueous extract is different qualitatively and quantitatively from the methanol extract used in this study and is expected to elaborate different biological activities [21-22]. Evaluation of the antiproliferative effect of Moringa oleifera on colon cancer cell lines showed that the ethanolic extract gave better activity on all cell lines than the aqueous extract [23]. There is scanty scientific information on the anti-proliferative and cytotoxicity studies of herbal concoctions of the quinine tree, traditionally used alongside Moringa oleifera to manage cancer and other diseases associated with oxidative stress in Kenya [15,25]. Furthermore, the present study targeted liver (hepatocellular carcinoma, Hep-G2) and muscular (rhabdomyosarcoma, RD) cell lines and cytotoxicity against Vero cell lines which have mostly been ignored. Studies on MO has been done by many research groups; the leaf extracts have been reported to induce apoptosis in KB carcinoma cells and inhibited lipid peroxidation as it scavenged free radicals and reduced oxidative stress [9]. In addition, different leaf extracts generated significant cytotoxicity effect on human multiple myeloma cultural cell lines and induced ROS production suggesting modulation of redox-sensitive mechanism [9, 26]. (Sreelatha and Padma, 2011; Parvathy and Umamaheswari, 2007).

Recently, Tiloke et al 2013 [24] showed that the antiproliferative effects of MO in A549 lung cells was as a result of increase in oxidative stress and DNA fragmentation thus inducing apoptosis. In separate investigations, the leaf extract inhibited the NF-kB signaling pathway and increased the efficacy of chemotherapy against human pancrease cancer cells, [27].

In the present study the anti-cancer activity and safety profiles of the active antioxidant extracts from M. oleifera and R. caffra used to treat cancer and related diseases were assessed to authenticate their traditional use. It was hypothesized that cell proliferation is aggrevated by oxidative stress in the cancerous cells and that extracts of M. oleifera and R. caffra with strong antioxidant activity would neutralize the free radicals generated by cell apoptosis and therefore reduce further cell multiplication.

II. Materials And Methods

2.1 Collection of Plants Materials

The leaves of *M. oleifera* and the stem bark of *R. caffra* were collected from Kuria County South Western Kenya (approximately 200 km from Kisumu city) on 23rd March, 2014. The traditional uses of medicinal plants were established during a survey done amongst the Luo and Kuria ethnic groups from Kuria county. The core study areas in Kuria district were in Kuria East and West constituencies. To ensure good data collection during the survey as prescribed in [28] a method of enquiring on diseases was preferred than enquiry on plant species. In the cancer disease category only *R. caffra* and *M. oleifera* plant species were reported among the Kuria, by one traditional medical practitioner, out of the eighteen interviewed. To complement the interviews, "guided-tours" were done with interviewees to observe plants cited and collect samples for botanical identification and authentication through laboratory studies. The collected plants were identified by a taxonomist from the University of Nairobi Herbarium, School of Biological Sciences where voucher specimens are deposited as Mutiso-MO-23/3/2014 and Mutiso-RC-23/3/2014 for *M. oleifera* and *R. caffra,* respectively.

2.2 Preparation of Plant Materials

The plant materials were air dried in the laboratory at room temperature for one week and milled into fine powder using an electric mill. 200g of *M. oleifera* leaf powder was extracted with 500 ml analytical grade

methanol (MeOH). The crude extract was obtained by filtering the resultant solvent and evaporating it using a rotary evaporator *in vacuo*. For *R. caffra* 200g of the stem bark powder were extracted with 500 ml mixture (1:1 v/v) of methanol and dichloromethane (CH_2Cl_2). Earlier studies showed that the MeOH extract of the leaves of *M. oleifera* and the extract of the stem bark of *R. caffra* obtained using 50% MeOH/CH_2Cl_2 exhibited optimal antioxidant activity (In press). Since oxidative stress is directly implicated in cancer and related ailments, these extracts were expected to show good anticancer activity.

2.3 Cell Lines

RD and Hep-G2 were donated by the Kenya Medical Research Institute (KEMRI) while Vero cell lines were obtained from the Department of Veterinary Services. The Vero cell lines were used as control cells [29].

2.4 Cell Culture Preparation and Maintenance

The cells were grown in media containing Dubelcos Minimum Essential Medium (DMEM) (Sigma-Aldrich, UK), 10% (v/v) Fetal Bovine Serum (FBS) (Invirogen, USA) and 2 mM L-glutamine, 1% penicillin/streptomycin (penstrep) (Invirogen, USA). The cells were maintained in a humidified incubator at 37 °C, 5% CO_2. Cells which had reached cellular confluence were trypsinized with 0.25% trypsin, 2 mM EDTA and re-suspended in the medium.

2.5 Anti-Proliferative Assay

Cytotoxicity assays were carried out as in Rakad and Jumaily (2010) [30]. Briefly, 0.005 g of the extracts were dissolved in 10 ml of DMEM medium (containing Dimethyl Sulfoxide (DMSO) of 1%) to give concentration ranging from 31.25 - 500 µg/ml. The cells were plated at a density of 1 X 10^4 cells/well in a 96-well plate and incubated for 24 hrs at 37 °C and 5% CO_2 prior to treatment with various concentrations of extracts, and incubated for 24, 48 and 72 hrs. Four replicate wells were prepared for each individual concentration and a negative control (media only) included. Crystal violet stain (50µl) was added to the wells and the plates incubated for 30 Min at 37 °C. Thereafter the cells were washed gently with distilled water three times and air dried. The Optical Density (OD) was recorded using anon ELISA reader (WILEX® Inc-Oncogene science USA) at 450 nm. The inhibitory rate of cell growth was calculated using the following formula;

Inhibition (%) = ((OD of control wells -OD of test wells)/OD of control wells)*100

The IC_{50} value was calculated using SPSS® Version 16 and the significant difference between control and sample means was assessed using student t-test on XLSTAT. A p value ≤ 0.05 was considered to be statistically significant

III. Results And Discussion

The aim of the current study was to study *M. oleifera* and *R. caffra* as possible sources of anticancer molecules, which may then be used in formulation of novel drugs for cancer treatment and management. The results of anti-proliferative effects of the MeOH extract of the leaves of *M. oleifera* are presented in table 1 and 2 while the activity of the extracts of the stem bark (50% MeOH/CH_2Cl_2) of *R. caffra* against the tested cell lines is summarized in table 3 and 4.

3.1 M. oleifera anti-proliferative activity against Vero cell lines

One important finding of this study is the low cytotoxicity displayed by the MeOH extract of *M. oleifera* leaves against Vero cells. After 72 hrs of exposure of the cells to high concentrations of the extract (500 µg/ml), the cytotoxic effect was found to be less than 50% (Table 1), this is an indication that the extract is less toxic to normal cells. Previous studies have found *M. oleifera* to be potentially non-toxic [31], validating its traditional use as a vegetable [6] water disinfectant [32] and as a medicinal herb.

Table 1: Percentage inhibition of Hep-G2, RD and VERO cell line by MeOH extract of *M. oleifera* leaves after 24, 48, and 72 hrs of exposure

Conc. (µg/ml)	**Inhibition %**								
	Hep-G2			**RD**			**VERO**		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
31.25	4.27	18.44	24.35	12.73	16.00	36.82	0.00	0.00	0.00
62.50	6.71	21.72	-	14.55	16.00	-	2.00	4.08	-
125.00	8.23	29.51	33.44	14.55	25.33	43.64	6.00	6.12	11.08
250.00	22.87	29.51	39.60	16.36	34.67	48.18	6.00	6.12	14.73
500.00	24.70	30.33	53.51	25.46	52.00	63.18	6.00	16.33	24.93
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

3.2 M. oleifera anti-proliferative activity against RD and Hep-G2 cell lines

The MeOH extract of *M. oleifera* leaves were found to have significant ($p \leq 0.05$) anti-proliferative activity against Hep-G2 and RD cell lines, corroborating with earlier findings [33]. However, RD cell lines were more sensitive to *M. oleifera* extract (IC_{50} = 0.017 mg/ml) than Hep-G2 (IC_{50} = 0.50 mg/ml), suggesting the presence of compounds that are more active against cell lines of sarcoma origin (Table 2). This data suggests that MeOH extract of *M. oleifera* leaves would contain compounds that have selective proliferative activity in different cancer cell lines. The anticancer activity of MeOH leaf extract of *M. oleifera* may in part be attributed to the presence of phenolic compounds in the plant [33]. In addition to its anticancer properties, *M. oleifera* is also a potent antioxidant [34-36] and portrays a wide spectrum antibiotic activity [37].

Table 2; IC_{50} values for Hep-G2, RD and VERO cell lines after 72 hrs exposure to *M. oleifera* leaf extracts (MeOH)

	IC_{50} (mg/ml)	***P* value**
Hep-G2	0.50	0.004
RD	0.17	0.005
VERO	3.78	-

3.3 R. caffra anti-proliferative activity against Vero cell lines

R. caffra stem bark extract (MeOH: CH_2Cl_2; 1:1) displayed cytotoxic effect of more than 50% at 500 g/ml against Vero cells after 48hrs of incubation, suggesting potential toxicity to non-cancerous cells at concentrations > 500 g/ml (Table 3).The toxicity exhibited by the R. caffra extract against Vero cells would be attributed to the presence of toxic alkaloids; characteristic of the genus Rauwolfia [38-39]. These findings suggest that the administration of R. caffra extract to patients using non-standardized pharmacological procedures as is the case in a traditional set-up may cause severe side effects to patients.

Table 3: Percentage inhibition of the growth of Hep-G2, RD and Vero cell line by extract of R. caffra stem bark (MeOH: CH_2Cl_2; 1:1) after 24, 48, and 72 hrs of exposure

Conc (µg/ml)	**Inhibition %**								
	Hep-G2			**RD**			**VERO**		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
31.25	15.85	13.60	13.04	0.00	0.00	23.41	0.00	1.724	23.78
62.50	17.68	15.64	19.57	0.00	5.33	37.05	2.00	-	34.19
125.00	17.68	26.13	16.09	3.64	16.00	49.77	2.00	10.35	35.00
250.00	21.65	20.76	27.39	10.91	28.00	51.59	4.00	13.45	37.46
500.00	31.71	34.82	42.17	18.18	50.67	61.14	20.00	32.59	51.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

3.4 R. caffra anti-proliferative activity against RD and Hep-G2 cell lines

The extract of *R. caffra* did not significantly affect the proliferation of RD and Hep-G2 cells (Table 4), which was contrary to what was observed for *M. oleifera* extract. A comparative analysis revealed RD cell lines to be more sensitive than Hep-G2 cell lines when exposed to *R. caffra* extract (Table 4); this was consistent with the result for *M. oleifera*. The low sensitivity of Hep-G2 cell lines to plant extracts has been observed from previous study by Mahavorasirikul *et al.,* (2010) [40].

Table 4; IC_{50} values for Hep-G2, RD and VERO cell lines after 72 hrs exposure to the extract of the stem bark extract of *R. caffra* (MeOH: CH_2Cl_2; 1:1)

	IC_{50} (mg/ml)	***P* value**
Hep-G2	0.89	0.059
RD	0.19	0.081
VERO	0.60	-

IV. Conclusion

Generally, MeOH extract of the leaves of *M. oleifera* was found to have substantial anti-cancer activity. It also displayed selective cytotoxicity against Hep-G2 and RD cell lines ($p \leq 0.05$) making it a suitable source of chemotherapeutic compounds and further supporting its use in traditional medicine to manage cancer related illnesses. It would be worthwhile to study the phytochemistry of the MeOH extract of *M. oleifera* and the resultant compounds evaluated for anticancer activity. The stem bark extract of *R. caffra* (MeOH: CH_2Cl_2; 1:1) however, exhibited high toxicity against Vero cells, suggesting potential toxicity to normal body cells; hence its use as a drug should be discouraged or restricted to topical application where there is less risk of developing severe side effects.

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Conflict of Interests

The authors declare no conflict of interest.

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