Cytotoxicity of 91 Kenyan indigenous medicinal plants towards human CCRF-CEM leukemia cells


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Article info

Article history:
Received 5 August 2015
Received in revised form 19 December 2015
Accepted 20 December 2015
Available online 22 December 2015

Keywords:
Albizia schimperiana
Solanum aculeastrum
Cancer
Kenyan flora
Leukemia
Traditional medicine

Abstract

Ethnopharmacological relevance: Plants from Kenyan flora are traditionally used against many ailments, including cancer and related diseases. Cancer is characterized as a condition with complex signs and symptoms. Recently there are recommendations that ethnopharmacological usages such as immune and skin disorders, inflammatory, infectious, parasitic and viral diseases should be taken into account when selecting plants that treat cancer.

Aim: The present study was aimed at investigating the cytotoxicity of a plethora of 145 plant parts from 91 medicinal plants, most of which are used in the management of cancer and related diseases by different communities in Kenya, against CCRF-CEM leukemia cell line.

Materials and methods: Extracts from different plant parts (leaves, stems, stem bark, roots, root barks, aerial parts and whole herb) were obtained by cold percolation using different solvent systems, such as (1:1 v/v) dichloromethane (CH₂Cl₂) and n-hexane (1), methanol (MeOH) and CH₂Cl₂ (2); neat MeOH (3), 5% H₂O in MeOH (4) and with ethanol (EtOH, 5); their cytotoxicities were determined using the resazurin reduction assay against CCRF-CEM cells.

Results: At a single concentration of 10 μg/mL, 12 out of 145 extracts exhibited more than 50% cell inhibition. These include samples from the root bark of Erythrina sacleuxii (extracted with 50% n-hexane-CH₂Cl₂), the leaves of Albizia gummifera, Strychnos usambensis, the stem bark of Zanthoxylum gilletii, Bridelia micrantha, Croton sylvaticus, and Albizia schimperiana; the root bark of Erythrina burttii and E. sacleuxii (extracted with 50% CH₂Cl₂-MeOH), the stem bark of B. micrantha and Z. gilletii (extracted using 5% MeOH-H₂O) and from the berries of Solanum aculeastrum (extracted with neat EtOH). The EtOH extract of the berries of S. aculeastrum and A. schimperiana stem bark extract displayed the highest cytotoxicity towards leukemia CCRF-CEM cells, with IC₅₀ values of 1.36 and 2.97 μg/mL, respectively. Other extracts having good activities included the extracts of the stem barks of Z. gilletii and B. micrantha and leaves of S. usambensis with IC₅₀ values of 9.04, 9.43 and 11.09 μg/mL, respectively.

Conclusions: The results of this study provided information related to the possible use of some Kenyan medicinal plants, and mostly S. aculeastrum, A. schimperiana, C. sylvaticus, Z. gilletii, B. micrantha and S. usambensis in the treatment of leukemia. The reported data helped to authenticate the claimed traditional medicinal uses of these plants. However, most plants are used in combination as traditional herbal concoctions. Hence, the cytotoxicity of corresponding plant combinations should be tested in vitro to authenticate the traditional medicinal practitioners actual practices.

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1. Introduction

The use of herbal medicine to manage various ailments is a common practice in developing countries, where most people...
depend on herbal drugs as the major form of treatment. Although cancers have been managed with herbal drugs as practiced in traditional medicine, there is very little scientific evidence on the efficacy of such herbs, since ethno-medical knowledge is normally held in secrecy by traditional herbalists, making it difficult to conduct any scientific investigations. It is therefore imperative to conduct biological assays on such herbs to establish their pharmacological efficacy and also possible toxic side effects. This strategy may also unveil novel bioactive molecules with anticancer potential.

The global burden for cancer has increased phenomenally in recent times and there was an estimated 14.1 million new cases of cancer in the world in 2012 (Ferlay et al., 2013). It was just recently that the cancer burden in Africa came into the center of scientific interest (Global Burden of Disease Cancer Collaboration, 2015). The development of drug resistance remains a major obstacle hindering successful cancer chemotherapy (Efferth et al., 2008, 2010; Kadioglu et al., 2015; Kuete and Efferth, 2015). These challenges among others have aggravated the continuous search for novel anticancer drugs. Natural sources of anticancer drugs, such as paclitaxel (Taxus brevifolia) and Vinca alkaloids (periwinkle plant, Catharanthus roseus) are examples for the value of traditionally used plants for modern drug development (Takimoto and Calvo, 2008). Previous studies have shown that extracts of African plants exhibited interesting cytotoxicity against sensitive (Choumessi et al., 2012a, 2012b; Dzoyem et al., 2013; Kuete et al., 2014a) and drug-resistant cell lines (Kuete et al., 2011, 2014b, 2014c; 2015a; 2015b). Plant extracts consisting of complex concoctions of compounds display more than one mechanisms of action on several targets and therefore might be better treatment options than synthetic drugs, because multifactorial activities may decrease the probability of emergence of resistant tumor clones (Efferth and Koch, 2011).

In Kenya, the cancer burden for the entire country is unclear; however, most practicing physicians have reported an increase in cancer cases among patients visiting local health facilities (Mutuma and Rugutt, 2006). Surgery, radiotherapy and chemotherapy remain gold standards in effective cancer management. However, the development of chemotherapeutic resistance may be a major factor for high relapse rates and mortality. Furthermore, it is difficult for scientifically untrained traditional practitioners to diagnose cancer. Recently, there are recommendations that the ethnomedicinal use of plants for immune and skin disorders, inflammatory, infectious, parasitic and viral diseases should be taken into account, when selecting plants used to treat cancer, since these reflect disease states may bear relevance to cancer or a cancer symptoms (Cordell et al., 1991; Popoca et al., 1998).

To date, a lot of progress has been made towards the discovery of effective anticancer drugs. This has involved the use of high through put technology such as computer-aided drug design but so far, screening of natural products has proved to be more promising (Kuete et al., 2015b). African flora has the potential to fight multidrug resistance of cancer (Kuete and Efferth, 2015).

Kenya has a rich biodiversity and rich folklore on use of medicinal plant for the treatment of various ailments including cancer, which has been well documented (Glover, 1996; Kaendi, 1997; Lindsay and Hepper, 1978; Maundu and Tengnäs, 2005; Kokwaro, 2009). Approximately 80% of Kenya’s population especially in 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. However, traditional medicine practice faces challenges such as quality of preparation, standardization, and unconfirmed safety profiles. Although many herbal remedies lay claim to anticancer effects, only a few have substantially been studied as alternative cancer therapies.

The traditional use of all plants investigated in the present study is compiled in Table S1 with special emphasis on the treatment of cancer and cancer-related symptoms. The four plants, which showed the highest cytotoxic activity and S. usambarensis are exemplarily described in more detail in the following paragraphs.

1.1. Zanthoxylum gilletii

Plants of this genus are used in Kenya to manage a number of ailments including cancer and related diseases. The stem bark of this plant is used to manage skin cancer (Ochwangi et al., 2014) and for reducing high blood pressure and coughs (Namukobe et al., 2011); the bark is chewed to treat stomach ache and toothache, joint pains, fever, rheumatism, sexually transmitted infections (STI) and for washing wounds (Kokwaro, 1993); the leaves are also used to ease coughs and are effective on gonorrhea and bilharzia (Gaya et al., 2013). This plant has only been evaluated for its antimalarial activity (Nkunya et al., 1990), however, this is the first report of its anticancer activity. The compounds that have been isolated from different parts of this plant that are most probably responsible for its bioactivities include; coagents alkaloids, aromatic and aliphatic amides, sterols and phenylpropanoids-lignans and coumarins, xanthophylls, phenolic acids, saponins, hydroxycinnamic acids (Adesina, 2005; Islam and Ahsan, 1997).

1.2. Strychnos usambarensis

The roots and leaves of S. usambarensis have antitumoral properties (Bassleer et al., 1982) and are used as arrow poison (Bassleer et al., 1982). Different plant parts have shown strong activity against P. falciparum (Wright et al., 1991; Frederich et al., 2004; Schmelzer and Gurib-Fakim, 2008a, 2008b); have antiamoebic (Wright et al., 1991) due to the presence of alkaloids (Schmelzer and Gurib-Fakim, 2008a, 2008b; Wright et al., 1991). This is the first report of the cytotoxic effects of the leaf extracts (50% MeOH in CH3Cl) of this plant.

S. aculeastrum different parts of S. aculeastrum are used to manage various ailments; the root bark, leaves and fruits are used to treat skin and cervical cancer (Ochwangi et al., 2014; Madhuri and Pandey, 2009; Koduru et al., 2006) the root bark is used for sexually transmitted bacterial diseases including gonorrhea and the berries to treat treating jigger infestations as well as acne (Agnew and Agnew, 1994; Kokwaro, 1993). The plant has been studied for its antitumor activity (Koduru et al., 2006); molluscicidal (Mkoji et al., 1989; Wanyonyi et al., 2003) and antimicrobial activities (Wanyonyi et al., 2003; Koduru et al., 2006; Steenkamp et al., 2007). The activities of different parts of this plant could be due to the presence of steroidal alkaloid glycosides (Wanyonyi et al., 2002, 2003) and saponins (Wanyonyi et al., 2002) characterized from these plant.

1.3. Albizia schimperiana

A. schimperiana is used extensively in Eastern Africa to manage a number of ailments. The roots are used to treat headache (Kokwaro, 1976), cure skin diseases and other pains (Kokwaro, 1993), drunk to treat pneumonia, tuberculosis, infertility of women and as an aphrodisiac (Bekele-Tesemma et al., 1993). The stem bark is used to treat cancer related diseases as warts (Kokwaro, 1976; Thulin et al., 1989). The leaves and bark to manage ulcerative lymangitis, colic, myiasis in animals (Tamiru et al., 2013) and the bark is used to treat malaria (Burkill, 1995; Beentje, 1994). Previous studies has shown that this plant elaborates
antimicrobial (Chhabra et al., 1981; Tariku, 2008; Samoylenko et al., 2009; Orwa et al., 2009); antihelmintic (Tariku, 2008; Eguale et al., 2011); antitrypanosomal (Cao et al., 2007); in vitro trypanocidal (Eguale et al., 2011); in vivo anti-trypanosomal activities (Tesfaye et al., 2015) and antiparasitic and cytotoxicity (Cao et al., 2007; Shugeng et al., 2007; Samoylenko et al., 2009); mainly attributed to the presence of Spermine alkaloids (budmunchiamines) and triterpenes (Endale et al., 1998; Eguale et al., 2006a, 2006b). Minimum cytotoxic effect were experienced by the CH<sub>2</sub>Cl<sub>2</sub> and MeOH extracts of the leaves of A. schimperi ana with IC<sub>50</sub> values of 225.6 and 184.1 μg/mL, respectively, on HL-cells (Nibert and Wink, 2011). The stem bark (50% CH<sub>2</sub>Cl<sub>2</sub> in MeOH) together with the spermene alkaloids, the budmunchiamines, showed cytotoxic effects against human cancer cells (Samoylenko et al., 2009).

1.4. Bridelia micrantha

Virtually, all parts of B. micrantha, that is, the stem bark, roots and leaves are used to manage cervical, breast, skin colorectal cancer (Ochwangi et al., 2014); the bark is used to bring full-term prolonged pregnancy (Brikensha, 2011); to treat skin ailments (Okello et al., 2010); venereal diseases, stomach ache, tapeworms and diarrhea in children (Kokwaro, 1993), dysentery (Hallam, 1979), pre-hepatic jaundice (Ssegawa and Kasenene, 2007); the stem bark is used as an abortifacient (Arnold and Gulumian, 1984; Van Wyk and Nigel, 2000, Steenkamp, 2003), to treat gastrointestinal ailments, paralysis and painful joints (Lin et al., 2002), to manage diabetes (Gbolade, 2009), as a remedy for severe epigastric pain, relief of headache and as purgative (Watt and Breyer-Brandwijk, 1962); bark and leaves are used for the treatment of syphilis (Ssegawa and Kasenene, 2007), the leaves are given to expel guinea worm (Abo et al., 2008; Nwaehujor and Udeh, 2011), as powerful purgative in cases of obstinate constipation and poisoning (Koné and Atindehou, 2008), for sore eyes (Watt and Breyer-Brandwijk, 1962), for management of diabetes mellitus (Abo et al., 2008), used against malaria-related fevers (Watt and Breyer-Brandwijk, 1962; Mabogo, 1990) and the roots are used as a remedy for severe epigastric pain, relief of headache and as a purgative (Watt and Breyer-Brandwijk, 1962). The following activities have been reported from this plant including hepatoprotective and antioxidant potentials (Nwaehujor and Udeh, 2011), antimicrobial (Hamill et al., 2003; Green et al., 2011; Okeleye et al., 2011; Traoré et al., 2015), in vitro anti-helicobacter pylori (Okeleye et al., 2011), anticonvulsant and sedative (Bum et al., 2011; Bum et al., 2012), anti-diarrheal (Lin et al., 2002), antiplasmodial (Clarkson et al., 2004), antiviral (Bessong et al., 2006), anethelmintic (Waterman et al., 2010), in vitro antiplasmodial activities (Bapela et al., 2014) and weak cytotoxic activities (Ajayoebo et al., 2006). The classes of chemical principles that have been reported from this plant previously include methyl salicylate (Ngueyem et al., 2009), phenolic compounds (Pegel and Rogers, 1968), alkaloids, flavonoids, steroids, tannins and saponins (Okeleye et al., 2011).

The current study was focused on leukemia, categorized as one of the ten most prevalent tumor types in Africa today (Global Burden of Disease Cancer Collaboration, 2015), with limited and traditional treatment options. Furthermore, these leukemia cell lines were selected as test model cells due to their high sensitivity towards cytotoxic agents as compared to tumor cell lines derived from solid tumor types. Using these cell lines, it is possible to observe inhibition effects even from extracts with modest activities that could otherwise be deemed inactive with solid tumor cells. The modern treatment options entailing the use of therapeutic monoclonal antibodies and target-specific small molecule inhibitors pose considerable challenges in terms of accessibility and affordability to majority of the population in developing countries. In addition, there are no proper medical policies or fee waivers that have been developed in these countries to cover the high expenses incurred in cancer treatment using the high-tech drugs. It is therefore imperative to develop novel and alternative treatment strategies to address these challenges that are leading to increasing cancer incidences and deaths especially from these countries. The purpose of the present study, was to establish the anti-leukemic potential of 145 extracts of 91 plants derived from Kenyan flora, preferably using human CCRF-CEM leukemia cells. Most of the investigated plants are used in indigenous medicine by different communities in Eastern Africa to manage cancer and related ailments. However, to the best of our knowledge, no scientific evidence is available on most of these plants regarding their cytotoxic potential.

2. Materials and methods

2.1. Plant material

The plant parts (leaves, stems, stem bark, roots, root barks, aerial parts and whole herb) of 91 plants species, most of which are used in the management of cancer and related diseases by different communities in Kenya (Table S1) were collected from different area in Kenya between August and September, 2014. The plants were identified by a taxonomist, Mr. Patrick Mutiso, from the University of Nairobi Herbarium, School of Biological Sciences (SBS) where voucher specimens are deposited (Table S1). The traditional uses of these medicinal plants were established in surveys carried out in earlier studies and by local traditional medical practitioners as summarized in Table S1.

2.2. Extraction

The plant materials were air dried in the laboratory at room temperature for one week and milled into fine powder using an electric mill at the Department of Chemistry, University of Nairobi. Five grams of the ground plant material were extracted with 100 mL of five different solvent systems including a mixture (1:1 v/v) of n-hexane-dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) (1), CH<sub>3</sub>Cl– methanol (MeOH) (2); neat MeOH (3), 5% MeOH–H<sub>2</sub>O (4) and with ethanol (EtOH, 5). The crude extracts were obtained by filtering the resultant solvent and evaporating it using a rotary evaporator in vacuo at reduced temperatures. The extracts were then conserved at 4 °C until further use.

2.3. Cell line

The human CCRF-CEM leukemia cell lines were maintained in RPMI 1640 (Life Technologies) supplemented with 10% FCS in humidified 5% CO<sub>2</sub> atmosphere at 37 °C. All experiments were done with cells in the logarithmic growth phase.

2.4. Resazurin growth inhibition assay

The in vitro response to drugs was evaluated by means of growth inhibition resazurin reduction assay (O’Brien et al., 2000) to assess the cytotoxicity of the test samples towards the human drug sensitive cancer cell lines (CCRF-CEM). The assay is based on reduction of the indicator dye, resazurin, to the highly fluorescent resorufin by viable cells. Non-viable cells rapidly lose the metabolic capacity to reduce resazurin and thus produce no fluorescent signal. Briefly, the crude extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted with RPMI medium to give an initial concentration of 20 μg/mL of various extracts. To determine the 50% inhibition from the dose response curves for most of the extracts that exhibited >70% cell inhibition including B. micrantha...
C. sylvaticus, gummifera, S. usambarensis; and micrantha. (Germany) 0.01% w/v in double-distilled water (ddH2O) was added to each well and the plates incubated for a further 4 h. Fluorescence was measured on an Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was done at least two times, with six replicate each. The viability was evaluated based on a comparison with untreated cells. IC50 values represent the sample’s concentrations required to inhibit 50% of cell proliferation and were calculated from a calibration curve by linear regression using Microsoft Excel. The resulting growth data represent the net outcome of cell proliferation and cell death.

3. Results and discussion

In the present work, 145 extracts from 91 plants from the Kenyan flora were tested for their cytotoxic effect with an ultimate aim of exploiting them as possible sources for leukemia therapy. The viabilities (% of untreated control) of CCRF-CEM cells after treatment with a fixed concentration of 10 μg/mL each are presented in Fig. 1 (Also see Supplementary data, Table S2). As can be seen, the majority of extracts were inactive or weakly active. Twelve plant extracts from nine plants inhibited the proliferation of CCRF-CEM cells by more than 50% following incubation for 48 or 72 h and therefore can be considered as being cytotoxic (Boik, 2001). The extracts included the root bark of E. sacleuxii extracted with 50% n-hexane-dichloromethane (CH2Cl2), the leaves of A. gummiifera, S. usambarensis; the stem bark of Z. gilletii, B. micrantha, C. sylvaticus, and A. schimperiana; the root bark of E. burttii and E. sacleuxii extracted with 50% CH2Cl2–MeOH, the stem bark of B. micrantha and Z. gilletii extracted using 5% MeOH–H2O and the berries of S. aculeastrum extracted with EtOH (Fig. 1).

The established anticancer drug, doxorubicin, was included into this cytotoxicity testing as a control drug. CCRF-CEM- cells revealed a viability of 65.7 ± 3.66% after treatment with 10 μg/mL doxorubicin. A detailed presentation of all screening data is given in Supplementary Table S2.

The four most cytotoxic extracts except for C. sylvaticus which was unavailable and S. usambarensis, tested at a fixed concentration of 10 μg/mL were then tested at different concentrations to obtain dose response curves to calculate 50% inhibition concentrations (IC50) (Fig. 2). The ethanol extract of the berries of S. aculeastrum and the stem bark of A. schimperiana (50% MeOH in CH2Cl2) exhibited the highest cytotoxic activity with IC50 values of 1.36 and 2.97 μg/mL, respectively. The other extracts that showed good activity included the extracts of the stem barks of Z. gilletii (5% MeOH–H2O), B. micrantha 50% CH2Cl2–MeOH and leaves of S. usambarensis (50% MeOH in CH2Cl2) with IC50 values of 9.040, 9.43 and 11.090 μg/mL.

More than one solvent systems from the following list; 50% CH2Cl2 in n-hexane, 50% MeOH in CH2Cl2, neat MeOH, 5% H2O in MeOH and EtOH were used for extraction of some of the plant materials to determine the variations of cell inhibition of the resultant extracts expected to vary in composition. The solvent systems that were frequently used in the experiments included; 50% MeOH in CH2Cl2 and 5% H2O in MeOH which have proved to be effective from previous studies carried out at the Chemistry Department, University of Nairobi, Natural Products Laboratory (Personal communication).

In the current study the most active extracts of some plant parts were those obtained using 50% MeOH in CH2Cl2 as elaborated in the stem bark of the following plants; C. sylvaticus Hochst (cell viability, 23.5%), B. micrantha (Hochst.) Baill (31.5%), Erythrina burttii Bakf.(47.5%), E. sacleuxii Hua (53.6%); the root bark of E. sacleuxii Hua (37.4%); aerial parts of S. usambarensis (42.9%), the leaves of A. gummiifera (JF. Gmel), C.A.Sm. (45.9%) and Senna dymobotyra (Fresen.) Irwin and Barneb (56.6%). The extracts that were most active when extracted with the more polar solvent systems including; 5% H2O in MeOH included; Z. gilletii (De Wild.) PG. Waterman (28.5%), Croton macrostachyus Del. (60.4%), and with ethanol; the berries of S. aculeastrum Dunali (0.8%). Most of the extracts obtained using the non-polar solvent, 50% CH2Cl2 in n-hexane exhibited poor activities, most probably, as they did not target the compounds responsible for the biological activities expected to be mainly different classes of flavonoids, alkaloids and terpenoids from the active plants. Furthermore, the plant parts used for traditional medicine seemed to be more active than those parts not used.

The root bark, leaves and fruits of the most active plant, S. aculeastrum, are used to treat skin and cervical cancer (Ochwangi et al., 2014; Madhuri and Pandey, 2009; Koduru et al., 2006). The anticancer activities of different parts of this plant are attributed to the presence of steroidal alkaloid glycosides (Wanyonyi et al., 2002, 2003) and saponins (Wanyonyi et al., 2002) characterized from this plant. Solanum species are known to elaborate these classes of compounds and their aglycones known to have different bioactivities including; anticancer, anticholesterol, antimicrobial, anti-inflammatory, antinoiceptive, antitussive and antipyretic effects, toxicity (Jiang et al., 2005; Milner et al., 2011). Other
compounds characterized from this the genus Solanum include: phenolics, flavonoids, sterols known to have different biological activities and hence could boost the activities of the major compounds; steroidal alkaloid glycosides and saponins (Amir and Kumar, 2004).

The present study involved the ethanol extract of the berries of S. aculeastrum against the drug sensitive human leukemia cells (CCRF-CEM) which exhibited interesting cell inhibition with an IC50 value of about 1.36 μg/mL. The results of this study are consistent with those of Koduru et al. (2006) in which the methanol extract of the berries of this plant showed antiproliferative activities against HeLa, MCF7 and HT29 with IC50 values between 17.1 and 41.9 μg/mL while the activities of the aqueous extract were lower, ranging between 27.9 and 48.5 μg/mL (Koduru et al., 2006).

The stem bark of A. schimperiana, which also showed good cell inhibition effect, is used widely in Eastern Africa to treat cancer related diseases as warfs (Thulin et al., 1989; Kokwaro, 1976). Previous studies has shown that this plant elaborates antimicrobial (Chhabra et al., 1981; Samoylenko et al., 2009; Tariku, 2008; Orwa et al., 2009); antihelmintic (Tariku, 2008; Eguale et al., 2011); antitrypanosomal (Cao et al., 2007); in vitro trypanocidal (Egual et al., 2011); in vivo antitrypanosomal activities (Tesfaye et al., 2015) and antiparasitic and cytotoxicity (Cao et al., 2007; Shugeng et al., 2007; Samoylenko et al., 2009); mainly attributed to the presence of spermine alkaloids (budmunchiamines) and triterpenes. The budmunchiamines from the genus Alibizia have demonstrated different bioactivities in previous studies including; in vivo antimalarial activity, suppressing Plasmodium berghei infection in mice after oral administration (Rukunga et al., 2007; Samoylenko et al. 2009), in vitro antimalarial activities against P. falciparum D6 (chloroquine-susceptible) and W2 (chloroquine-resistant) strains with IC50 values ranging from 120–270 ng/mL, strong antileishmanial activities with 5,14-dimethylbudmunchiamine L1 and 6-hydroxy-5-normethylbudmunchiamine K exhibiting equipotent activities with the standard drug, pentamidine, and 5-normethylbudmunchiamine K being more potent. The two spermine alkaloids ca. 5,14-dimethylbudmunchiamine L1 and normethylbudmunchiamine K also exhibited significant in vitro antimicrobial activities against a panel of microorganisms and moderate cytotoxic activities (Samoylenko et al., 2009). The budmunchiamines L4 and L5 isolated from Pithecolobium saman (Wiesner et al., 1952, 1968) and Alibizia species from both India (Mar et al., 1991; Pezzuto et al., 1992; Misra et al., 1995) and Kenya (Rukunga and Waterman, 1996a, 1996b) exhibited mild activities against plasmspin II, with IC50 values of 14 and 15 μM, respectively (Ovenden et al., 2002).

Minimal cytotoxic effects were experienced by the CH2Cl2 and MeOH extracts of the leaves of A. schimperiana with IC50 values of 225.6 and 184.1 μg/mL, respectively, on HL-cells (Nibret and Wink, 2011). Samoylenko et al. (2009) demonstrated the potential of the stem bark (50% CH2Cl2 in MeOH) together with the spermine alkaloids, the budmunchiamines, against different human cancer cells. However, the focus of the previous study was on the cytotoxicity of the characteristic compounds of this plant. The current study has generated additional data by determining the IC50 value of the stem bark (50% CH2Cl2 in MeOH) of this plant not established before. The observation that the cytotoxicity of the extracts was higher than that of constituent compounds as shown in previous studies could either be attributed to the synergistic phenomena of the constituent components or to the fact that the most active compound is minor and was not isolated. Further, studies should be carried out to isolate the minor budmunchiamines in order to establish their cytotoxicities. The functionalities on the skeletal structures of these spermine alkaloids with moderate cytotoxic effects should be modified towards increasing their cytotoxic potencies. The effect of opening up the macrocyclic ring on the cytotoxic activities should also be established. The hydroxyl groups on the macrocyclic ring, which adversely influenced the biological activities of the budmunchiamines should be modified to obtain different analogs expected to show varied activities.

The present investigation was designed as an exploratory screening study. Using 91 Kenyan plants, we analyzed a large panel of medicinal plants used in traditional Kenyan medicine. The aims were first to give an overview of the traditional uses of these plants with a focus on cancer and cancer-related symptoms. As indigenous knowledge on traditional plants is handed over in oral form from generation to generation, there is a danger that this knowledge or parts of it will get lost over time. Therefore, it is of utmost importance to document the traditional uses of medicinal plants. A second aim of this study was to screen these plants for their cytotoxic activity towards human CCRF-CEM leukemia cells. We have chosen leukemia cells, because leukemia are very frequently more sensitive to cytotoxic agents than most other tumor types. Therefore, they are better suited for initial compound screenings than tumor cell lines from other tumor origin. This was also the strategy of the National Cancer Institute, U.S.A., which screened compounds for many years with the murine leukemia cell line P388, before they enlarged their screening panel to cell lines of other tumors types. The present study represents a starting for subsequent investigations. The extracts which showed promising cytotoxicity will be investigated in more detail concerning (1) their activity towards cell lines from different types of solid cancers, (2) their activity towards cell lines resistant to established anticancer drugs, including multidrug-resistance phenotypes, and (3) their active chemical constituents by means of bioactivity-guided fractionation and isolation.

Authors' contribution

L.K.O carried out the experiments; L.KO, wrote the manuscript, J.O.M and V.K edited the manuscript. M.E.M, S.H and T.E. designed the bioassay experiments; B.M.G, R.M and V.M.M, F.K- K., contributed to extraction of plant materials. G.O contributed in reading bioassay results in Tecan scanner. T.E. supervised the work, provided the facilities for the study, edited the manuscript. All authors read the manuscript and approved the final version.

Conflict of interests

The authors declare not to have a conflict of interest.

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Acknowledgment

The authors wish to thank Mr. Patrick Chalo Mutiso for the identification and collection of the plant materials, Ms. Christine Koeppel for assistance with biological screening. L.K.O is grateful to International Science Program, Uppsala University, Sweden (ISP)-KEN-02 project for the three months’ sponsorship in Germany to carry out this study.


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