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Phytochemical Screening of Medicinal Plants of the Kakamega Country, Kenya Commonly Used against Cancer

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Abstract

Phytochemical constituents of 35 selected anticancer medicinal plant extracts were analyzed by thin layer chromatography (TLC) for alkaloids, anthraquinones, xanthines, valepotriates, cardiac glycosides, flavonoids, essential oils, coumarins, lignans, saponins and arbutin compounds. These plants sourced from Kakamega tropical rain forest have been previously reported to be used for cancer treatment in the Kakamega County, Kenya. The medicinal plants investigated were *Harungana madagascariensis* Lam. ex poir, *Fuerstia africana* T.C.E. Fr., *Sida rhombifolia* L., *Zanthoxylum rubescens* Hook. f, *Bridelia micrantha* (Hochst.) Baill, *Juniperus procera* Endl., *Tragia brevipes* Pax, *Phyllanthus sapialis, Conyza sumatrensis, Momordica foetida* Schumach., *Synsepalum cerasiferum* Synonym: *Afrosersalisia cerasifera* (Welw.) Aubrev, *Aloe volkensii* Engl, *Aeschynomene abyssinica* (A. Rich.) Vatke, *Futumia africana* Benth. *Cyphostemma serpens* (A. Rich), *Ipomoea cairica* (L.), *Spathodea campanulata* P. Beauv. *ssp. nilotica* (Seem), *Abrus precatorius* L. *ssp africanus* Verdc., *Triumfetta rhomboidea* Jacq., *Psydrax schimperiana* (A. Rich), *Ficus thonningii, Rotheca myricoides* (Hochst. Steane and Mabb), *Croton macrostachyus* Delile, *Vernonia lasiopus* O Hoffin, *Albizia gummifera* (J.F. Gmel.), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman, *Microglossa pyrifolia* (Lam.) Kuntze, *Senna didymobotyra* (Fresen.) Irwin and Barneby, *Trichilia emetica* Vahl, *Entada abyssinica* Steud.ex A.Rich., *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax, *Ocimum gratissimum* L. Suave wiild O. tomentosum oliv., *Prunus africana* (Hook.f.) Kalkman, *Phyllanthus fischeri* Pax and *Olea hotch spp. Hochstetteri*. The phytochemical distribution in the 35 plants included: 71.4% alkaloids, 57.1% anthraquinones, 94.2% xanthines, 82.8% valepotriates, 94.2% cardioactive glycosides, 82.8% flavonoids, 77.1% essential oils, 85.7% coumarin drugs, 68.5% lignans, 80% saponins and 62.85% arbutin drugs. Our findings provided evidence that crude organic and inorganic solvent extracts of these tested plants contain medicinally important bioactive compounds and provide a rational basis for their use in traditional medicine.

Keywords: Phytochemicals; Alkaloids; Flavonoids; Saponins

Introduction

A majority of important bioactive compounds that produce desirable physiologic activity have been derived from plants. These compounds include but are not limited to phenolic compounds, alkaloids, tannins and flavonoids [1]. These compounds are diverse secondary metabolites that can be utilized for drug development in human and veterinary medicine [2]. Plant products derived from barks, leaves, flowers, roots, fruits or seeds have been part of phytomedicines from the ancient of days [3]. Lead compounds with great pharmacological features have been synthesized from these plants [4]. In the present investigation, the phytochemical constituents of 35 selected herbal extracts were analyzed for alkaloids, anthraquinones, xanthines, valepotriates, cardiac glycosides, flavonoids, essential oils, coumarins, lignans, saponins and arbutin drugs by thin layer chromatography. These medicinal plants have been previously reported to have anticancer properties [5].

Materials and Methods

Extract preparation

Organic and aqueous extracts were prepared using dichloromethane, methanol and water. Ten grams of powdered plant material was extracted by cold maceration in a dichloromethane: methanol mixture at a ratio of 1:1 for 72 h at room temperature. The extract was reduced in a rotavapor and thereafter in an oven at 40°C. The yield of each extract was determined and the extract preserved at 4°C. For aqueous extraction, the plant powder was macerated in distilled water at a ratio of 1:6 (w/v). The suspension was rotated on a shaker for 24 h at room temperature and filtered using cotton wool and Whatman filter paper No 1. The aqueous extracts were freeze dried and the resultant lyophilized residues stored at room temperature.

Thin layer chromatography (TLC)

The phytochemical analysis of extracts of commonly used medicinal plants was carried out using TLC as described by Wagner. Extract aliquots of 2.5 mg were dissolved in 500 µL of methanol. Silica gel 60F 254-aluminium foils (0.2 mm layer thickness) (Millipore Corporation, Germany) were used. The TLC plates were fan-dried shortly before applying the substances with starting points in a height of 1-1.5 cm. The distance between the starting points was 0.5 cm and 1 cm to the plate boundary. It proved reasonable to apply the substances not only as points, but also as bar marks. The glass capillaries used to apply the solution corresponded to 1.2 cm liquid column of 5 µL. The

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plate was well dried after every application. The developing chamber with super plasticizer was lined with filter paper (the super plasticizer moves faster). The development of the TLC plates took place, if the chamber was saturated. The super plasticizer was completely flashed off, if the plate was developed by fan-drying or putting them in a drying cabinet before detection and taking chromatogram pictures.

Spray solutions

Spray solutions were prepared and used for the various analyses. Glass spray heads were used, if the solution contained sulphuric acid. These solutions were as follows:

Anisic aldehyde-sulfuric acid-reagent: Anisic aldehyde (0.5 mL) was slowly mixed with 10 mL acetic acid, 85 mL MeOH and 5 mL conc. sulphuric acid in that order. The solution was kept in the refrigerator.

Dragendorff reagent: Basic bismuth nitrate (1.7 g) and 20 g tartaric acid were suspended in 40 mL water. Potassium iodide (16 g) was dissolved in 40 mL water and added to the bismuth nitrate suspension. The resultant liquid was stirred for 1 h and filtrated. The stock solution was kept several days in brown glass bottles in the refrigerator and protected from light. The spray solution was prepared by mixing 5 mL stock solution with 15 mL water.

Ethanolic KOH reagent: A 5% solution of KOH in EtOH 50% (V/V) was prepared, which comprised of 2.5 g KOH dissolved in 25 mL water and 25 mL ethanol.

Iodine-platinate reagent: Potassium hexachloroplatinatein (0.15 g) was dissolved in 50 mL water, while heating. Then, 50 mL of a 6% potassium iodine solution were added and the solution kept in the refrigerator.

Potassium permanganate-sulfuric acid reagent: $KMnO_4$ (1 g) was carefully dissolved in 30 mL conc. sulphuric acid and cooled with ice to avoid the generation of explosive manganheptoxide.

Natural substance-PEG reagent: Solution A: A 1% solution of natural substance reagent A (diphenylboronic acid-aminoethyl ester) in MeOH was prepared and was kept in the refrigerator. **Solution B**: A 5% solution of polyethylene glycol 4000 in EtOH was prepared. Solutions A and B were sprayed one after the other onto the TLC plate. The PEG solution increased the detection sensivity.

Hydrochloric acid-glacial acetic acid reagent: Fourty milliliters conc. hydrochloric acid were mixed with 10 mL glacial acetic acid.

Vanillin sulfuric acid reagent: Solution A: A 5% solution of conc. sulfuric acid in EtOH was prepared (5 mL acid mixed with 95 mL ethanol). **Solution B**: A 1% solution of vanillin in EtOH was prepared (1 g vanillin mixed with 99 mL ethanol). Solution A was first sprayed onto the TLC plate and immediately thereafter, solution B was sprayed.

Methanolic sulphuric acid: Methanolic sulphuric acid (10% V/V) was prepared by mixing 10 mL H_2SO_4 with 90 mL methanol.

TLC analyses

A total of 35 plant extract samples were investigated, 10 samples per plate with bands of 10 mm length together with their corresponding references for each compound class.

Alkaloids: The reference compounds used included: Cinchonidin, cinchonin and chinidium dissolved in 500 µL of methanol applied at a final volume of 5 µL at a point. A super plasticizer of toluol/EtOAc/DEA: 5/4/1 was used with a running height of 12 cm. The plate was dried to remove DEA (fan-dry or put into a drying cabinet at 100°C). The chromatogram was evaluated under UV light (254 or 365 nm) and afterwards sprayed with 10% methanolic sulphuric acid (V/V) onto the plate. The chromatogram was evaluated again under UV light (365 nm) and sprayed with iodine-platinate-reagent onto the plate and thereafter evaluated in daylight.

Anthraquinones: The reference solutions were 1 mg aloin and 1 mg franguline dissolved in at 5 µL. Super plasticizer consisting of EtOAc / MeOH / H_2O 50 / 8.5 / 6.5 was used with a running height of 15 cm. The plate was dried (max. 10 min) and immediately sprayed with freshly prepared solution of nitrosodimethyl aniline in pyridine (0, 1%). The chromatogram was immediately evaluated. Ethanolic KOH reagent was sprayed onto it afterwards and put into a 100-105°C warm drying cabinet for 15 min. The plate was evaluated in daylight and in UV light (365 nm).

Xanthines: The reference solutions were caffeine, theophylline and theobromine dissolved in 300 µL methanol applied at a volume of 5 µL per point. Super plasticizer consisting of toluol / isopropanol / NH_3 conc. 15 / 30 / 5 was used with a running height of 15 cm. The dry TLC plate was evaluated under UV light (254 nm) and sprayed with potassium permanganate-sulfuric acid reagent. It was subsequently fan-dried and put into a 50°C warm drying cabinet for 15 min. Dragendorff reagent was sprayed onto the TLC plate afterwards and evaluated immediately in daylight.

Valepotriates: The reference solution was Valmane®. The sugar coat of one tablet Valmane® was removed and half pulverized in a mortar. Ether (1.5 mL) was added to this powder in an Eppendorf vessel. An aliquot of 10 µL was applied per point as bands 15 mm length. Super plasticizer of toluol / EtOAc: 37.5 / 12.5 was used with a running height of 15 cm. The dry TLC plate was evaluated in UV light (254 nm) and sprayed with hydrochloric acid-acetic acid reagent. The plate was heated under supervision in a 110°C warm drying cabinet for 10 min. The evaluation occurred in daylight and in UV light (365 nm). The zone's colours deepened while heating the TLC plate. This was the reason, why continuous supervision was important.

Cardioactive glycosides: The reference solutions used were 2 mg digitoxin and 1 mg gitoxin mixed in 3 mL MeOH and 2 ml ether. An aliquot of 20 µL of this mixture was applied per point. Super plasticizer of EtOAc / MeOH / H_2O 44 / 5.5 / 4 was used with a running height of 15 cm. For detection and evaluation, conc. H_2SO_4 was sprayed onto the dried chromatogram and evaluated in daylight. After putting it into a 100°C warm drying cabinet for 3-5 min, it was evaluated under UV light (365 nm).

Flavonoids: The reference solution consisted of 1 mg of rutin, chlorogenic acid and hyperoside dissolved together in 1 mL MeOH. Also, 1 mg of caffeic acid dissolved in 1 mL MeOH was used. Aliquots of 25-30 µL of the herbal drug extracts and reference solutions were applied as bands of 10 mm length. Super plasticizer of EtOAc / formic acid / acetic acid / H_2O 50 / 5.5 / 5.5 / 13.5 was used. The first three components were mixed and water was slowly added while shaking the mixture with a running height of 15 cm. The super plasticizer was completely fan-dried or dried by putting the plate into a 100°C warm drying cabinet. The chromatogram was evaluated in UV light (254 and 365 nm). It was controlled after 15-20 min. Afterwards, natural substance-PEG reagent was sprayed onto the plate and evaluated in daylight.

Essential oils: A solution of 5 mg or µL anethole, carvone, 1, 8-cineol (eucalyptol), eugenol, menthol and thymol in 1.0 mL toluol

was used as reference solution. Super plasticizer of toluol / EtOAc 93 / 7 was used with a running height of 6 cm. After evaluating the dry TLC plate in UV light (254 and 365 nm), vanillin-sulfuric acid reagent was sprayed onto it and heated in a 110°C warm drying cabinet for 5-10 min under supervision. The evaluation occurred in daylight.

Coumarin drugs: The reference solution was caffeic acid dissolved in methanol with super plasticizer of toluene-ether 1:1 saturated with 10% acetic acid and a running height of 15 cm. Detection and evaluation occurred under UV light (365 nm) without further chemical treatment.

Lignans: The reference solution of rutin, chlorogenic acid and hyperoside dissolved in methanol was used with super plasticizer consisting of chloroform-methanol-water (70:30:4) and a running height of 15 cm. Detection and evaluation was done after drying the plate and spraying with PEG reagent and evaluating under UV light (365 nm).

Saponins: As reference solution, aescin dissolved in methanol with super plasticizer of chloroform-acetic acid-methanol-water; 60:32:12:8 with a running height of 15 cm was used. Detection and evaluation was done after drying the plate in a warm cabinet, watching under UV light (254 and 365 nm) and spraying with anisaldeyde-sulphuric acid reagent.

Arbutin drugs: The reference solutions included arbutin and rutin in 300 µL methanol with a super plasticizer of ethyl acetate-methanol-water (100/13.5:/10) and a running height of 15 cm. After drying the plate in a warm cabinet and evaluating under UV (256 and 354 nm), natural products polyethylene glycol reagent (NP/PEG) was sprayed and evaluated under UV light (365 nm).

Results

Phytochemical screening results

TLC results of phytochemical constituents of 35 medicinal plants were summarized in Table 1 representing the designated sample ID, the botanical name of each medicinal plant and the various phytochemical constituents, i.e., alkaloids, anthraquinones, xanthines, valepotriates, cardiac glycosides, flavonoids, essential oils, coumarin drugs, lignans, saponins and arbutin drugs. The results revealed the presence of numerous medically active compounds in the plant extracts. A synopsis of the frequency of all compounds detected in our set of 35 plants has been compiled according to the chemical class they belong to. As can be seen in Figure 1, xanthines, cardioactive glycosides and coumarins were the most frequent compounds (85.7% to 94.2%), while lignans, arbutin drugs, and anthraquinones were the least frequent phytochemicals (57.1% to 68.5%). All other chemical classes were present in intermediate frequencies. As can be seen in Figure 2, Representative TLC chromatograms of phytochemicals of medicinal plants from the Kakamega County, Kenya.

(a) Alkaloids: The predominantly light blue fluorescence of alkaloids was intensified by treatment with 10% methanolic sulphuric acid. Other alkaloids showed bright yellow and yellow green florescence.

(b) Anthraquinones: The bands showed characteristic yellow and blue fluorescence. All major compounds such as aloins or aloinosides showed quenching in UV light (254 nm). Treatment with KOH reagent intensified the yellow fluorescence of aloin and aloinosides as well as the blue fluorescence of aloe resins.

(c) Xanthines: Chromatograms showed red, blue and green fluorescence at UV light (360 nm).

(d) Valepotriates: The ingredients of Valmane® reference solution showed Rf-values of 0.70 for valtrate/isovaltrate, 0.60 for didrovaltrate, 0.55 for acevaltrate, and 0.40 forIVHD. The chromatogram showed blue and brown fluorescences.

(e) Cardioactive glycosides: After spraying with conc. sulphuric acid and heating for 1-3 min at 100°C, blue, brown, green and yellowish fluorescences appeared at 365 nm and appeared brown or blue in normal daylight.

(f) Flavonoids: The chromatograms showed yellow-orange or yellow-green and blue fluorescences. Spraying with PEG reagent strengthened the fluorescence signals.

(g) Essential oils: At a wavelength of 254 nm UV light, the fluorescence was quenched and bands appeared as dark zones against a light green fluorescent background of the TLC plate. After spraying with vanillin sulphuric acid essential oil, strong blue, green, red and brown stainings appeared.

(h) Coumarin drugs: The chromatograms showed characteristic bright blue, blue-green, yellow green and violet-blue fluorescence of coumarins at a wavelength of 365 nm UV light.

(i) Lignans: The chromatograms showed blue and blue-green fluorescence zones under UV light of 365 nm. The blue fluorescence was strengthened after spraying with PEG reagent.

(j) Saponins: The chromatograms showed grey blue, yellow brown and red fluorescence bands at UV light (365 nm) after spraying with anisaldeyde-sulphuric acid reagent.

(k) Arbutin drugs: Distinct grey and blue zones were observed in Figure 2.

Discussion

In the present study, we investigated the phytochemical constituents of 35 selected medicinal plants used in the Kakamega County, Kenya for the treatment of cancer. Phytochemical analyses of the present study revealed the presence of bioactive constituents known to possess important pharmacological effects [6]. Analyses of the plant extracts revealed the presence of alkaloids, anthraquinones, xanthines, valepotriates, cardioactive glycosides, flavonoids, essential oils, coumarins, lignans, saponins, and arbutin drugs. Different reference compounds were used as controls. In total, 71.4% of the medicinal plants contained alkaloids, 57.1% anthraquinones, 94.2% xanthines, 82.8% valepotriates, 94.2% cardioactive glycosides, 82.8% flavonoids, 77.1% essential oils, 85.7% coumarin drugs, 68.5% lignans, 80% saponins, and 62.85% arbutin drugs. These results show that the majority of the selected anti-cancer medicinal plants have a wide range of biologically important phytochemicals with cardioactive glycosides and xanthines as most abundant compounds. Phenolic compounds such as flavonoids have been previously shown to have anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, and cardiovascular protective activities [7]. Flavonoids in plants comprise a vast array of biologically active compounds which have been used in traditional medicine for many years and have majorly antioxidant and antiproliferative effects especially against chronic inflammatory and allergic diseases, breast cancer and coronary artery disease [8]. Ogawa et al. has reported evidence of flavonoids having antimutagenic activity of quercetin that was shown to inhibit the mutagenic activity of benzo[a]pyrene, a polyaromatic hydrocarbon carcinogen [9]. Flavonoids have also been found to be an active antimicrobial substance against a variety of microorganisms *in vitro*

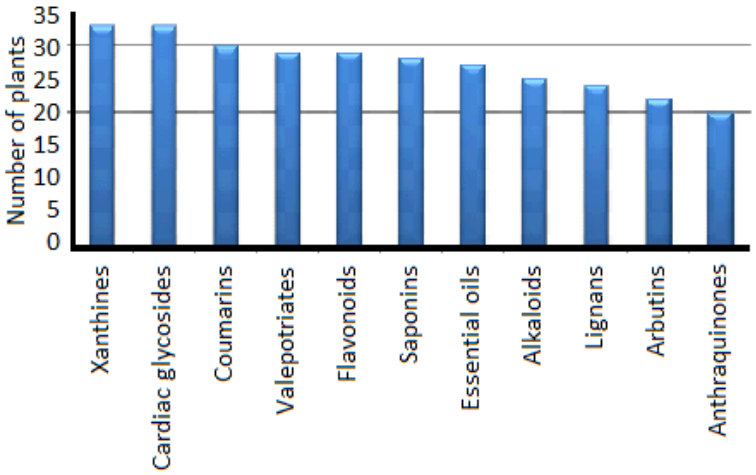

Figure 1: Frequency of phytochemical constituents: 71.4% alkaloids, 57.1% anthraquinones, 94.2% xanthines, 82.8% valepotriates, 94.2% cardioactive glycosides, 82.8% flavonoids, 77.1% essential oils, 85.7% coumarin drugs, 68.5% lignans, 80% saponins, and 62.85% arbutin drugs.

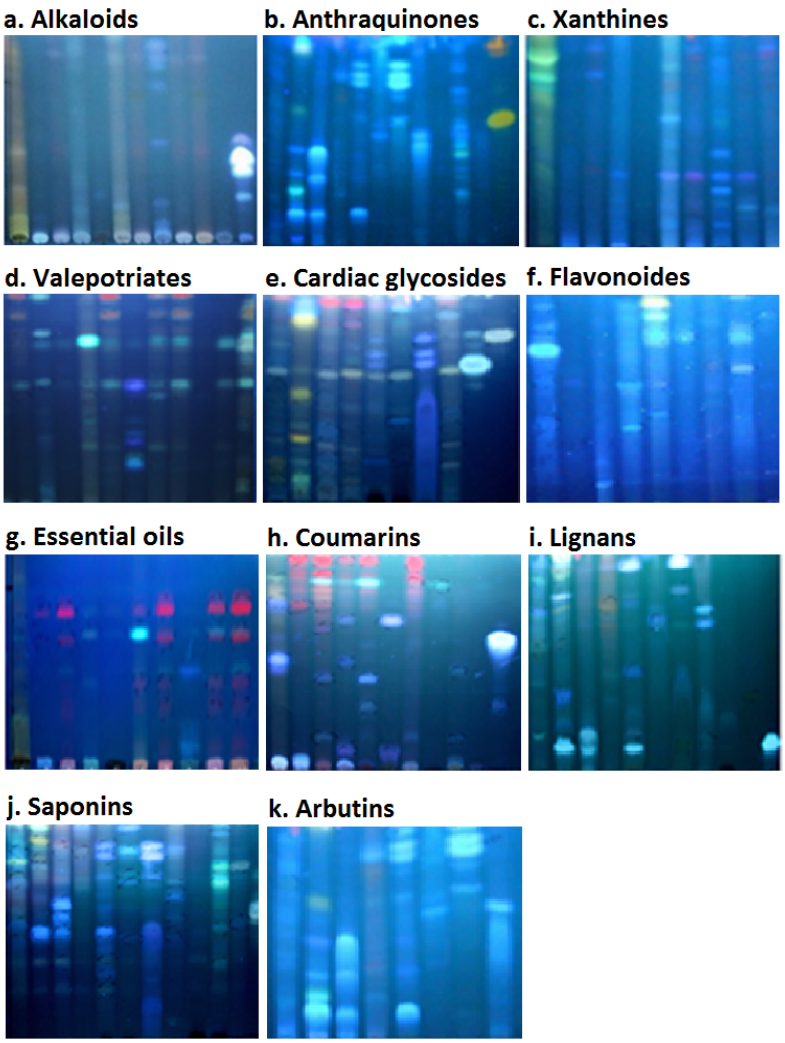

Figure 2: Representative TLC chromatograms of phytochemicals of medicinal plants from the Kakamega County, Kenya. (a) Alkaloids, (b) Anthraquinones, (c) Xanthines, (d) Valepotriates, (e) Cardioactive glycosides, (f) Flavonoids, (g) Essential oils, (h) Coumarin drugs, (i) Lignans, (j) Saponins, (k) Arbutin drugs.

Sample ID	Plant Species (Botanical name)	Phytochemicals Constituents (+: indicating presence and –: indicating absence)										
		Alkaloids	Anthraquinones	Xanthines	Valepotriates	Cardioactive Glycosides	Flavonoids	Essential Oils	Coumarin Drugs	Lignans	Saponins	Arbutin Drugs
1	*Harungana madagascariensis* Lam. ex poir (Stem bark)	++	–	++++	++++	+	–	+++	++++	–	–	–
2	*Fuerstia africana* T.C.E. Fr. (Whole plant)	–	+	++	++	++++	++	++	+++	++	++++	+++
3	*Sida rhombifolia* L. (Leaves)	+	–	++++	++++	++++	++	++	+++	+	++	+
4	*Zanthoxylum rubescens* Hook. f (Stem bark)	++++	+	++	++++	++++	++	++	–	+	+++	–
5	*Bridelia micrantha* (Hochst.) Baill (Stem bark)	+	–	–	–	+	–	–	–	–	–	–
6	*Juniperus procera* Endl. (Stem bark)	++++	+	++++	++++	++++	++	++	+++	++	++	++
7	*Tragia brevipes* Pax (Leaves)	++	–	+++	++	++++	++	+++	++++	++	++	+
8	*Phyllanthus sapialis* (Leaves)	++++	+	++++	+++	++++	+++	–	++++	++++	++++	+++
9	*Conyza sumatrensis* (Retz.) E.H Walker (Leaves)	+	–	+++	++++	++++	++	++++	+++	++	+++	+++
10	*Momordica foetida* Schumach. (Whole aerial plant)	++	–	++++	++++	++++	+	++++	++++	+	+++	–
11	*Synsepalum cerasiferum* Synonym: *Afrosersalisia cerasifera* (Welw.) Aubrev (Stem bark)	–	–	–	–	+++	+	–	–	+	+++	+
12	*Aloe volkensii* Engl. (Leaves)	+	+	++++	–	++++	++	++	++	–	++	–
13	*Aeschynomene abyssinica* (A. Rich.) Vatke (Leaves)	+	–	++	++++	++++	+++	++	+++	–	+++	+
14	*Futumia africana* Benth. (Leaves)	++	–	++	++++	++	–	+	+	–	–	–
15	*Cyphostemma serpens* (A. Rich) (Roots)	+	+	++	++	++++	++++	+	++	++++	++++	++
16	*Ipomoea cairica* (L.) (Leaves)	++	–	++++	+++	++++	+	+++	+++	–	–	–
17	*Spathodea campanulata* P. Beauv. *ssp. nilotica* (Seem) (Stem bark)	–	–	+++	++	++++	+	++++	++	–	+	–
18	*Abrus precatorius L. ssp africanus* Verdc. (Roots and seeds)	++	++	++	++	+++	+++	++	+++	++	++++	+++
19	*Triumfetta rhomboidea* Jacq. (Leaves)	+	++	++	+++	++++	+++	++	+++	+++	++	+++
20	*Psydrax schimperiana* (A. Rich) (Stem bark)	++	++	++	++	+++	+	+	++	+	+++	++
21	*Ficus thonningii* (Leaves and stem bark)	++++	+	++	+++	++	–	++	+++	–	+	–
22	*Rotheca myricoides* (Hochst. Steane and Mabb.) (Whole plant)	+	+	+	+	+++	++++	–	+	+++	+++	+
23	*Croton macrostachyus* Delile (Stem bark)	–	–	+	++	+	–	++	+	+	–	–
24	*Vernonia lasiopus* O Hoffin (Stem bark)	–	–	++	–	–	+	–	–	–	–	–

25	*Albizia gummifera* (J. F. Gmel.) (Leaves and Stem bark)	+	_	++++	++++	++++	++	++	++	+++	+++	++
26	*Zanthoxylum gilletii* (De Wild.) P. G. Waterman (Stem bark)	++++	++++	++++	++++	++++	++++	++	+++	++++	++++	++++
27	*Microglossa pyrifolia* (Lam.) Kuntze (Leaves)	_	++++	++++	+++	++++	+	++++	+++	++	++++	++++
28	*Senna didymobotyra* (Fresen.) Irwin and Barneby (Leaves)	+++	+	++++	++++	++++	+	++++	++++	+	+	+++
29	*Trichilia emetica* Vahl (Stem bark)	–	++++	++++	+++	+++	++++	++	++++	+++	++++	++++
30	*Entada abyssinica* Steud. ex A. Rich. (Stem bark)	++	++	++++	++++	++++	+	++	++++	+	++++	+
31	*Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst. kraus) Pax (Stem bark)	–	+++	++	+	+++	+++	_	++	+++	++++	+++
32	*Ocimum gratissimum* L. Suave wiild O. tomentosum oliv. (Leaves)	++	++	++++	++++	++++	++	++++	+++	+++	+++	+
33	*Prunus africana* (Hook.f.) Kalkman (Stem bark)	–	–	++	++	_	_	+	+	_	_	_
34	*Phyllanthus fischeri* Pax (Leaves and roots)	++	++	++++	_	+++	++++	_	++	_	++++	_
35	*Olea hotch spp.* Hochstetteri (Stem bark)	–	+	++++	_	+	+	_	_	+	+++	+

Table 1: Thin layer chromatography of medicinal plants commonly used for cancer treatment in the Kakamega County, Kenya.

with their activity postulated to be due to their ability to complex with extracellular and soluble proteins of bacterial cell wall [10]. Besides they have shown effectiveness as antioxidants and strong anticancer activities [11].

Essential oils do not represent a chemically consistent class of substances. They are all characterized by high volatility and aromatic smell. Most of the molecules consist of mono- and sesquiterpenes. Phenylpropane base units and sulfur and nitrogen containing substances (for example anthranilic acid esters) were also found. A plant's essential oil is composed of many individual components (Wagner and Bladt). α-Cadinol, β-elemene and α-humulene have been shown to be cytotoxic against tumor cell lines [12].

Coumarin is a natural substance that has shown to have *in vivo* anti-tumor activity. A recent study has shown that 7-hydroxycoumarin inhibited the release of cyclin D1, which is overexpressed in many cancer types [13]. Many compounds derived from coumarin have numerous therapeutic applications, which include photochemotherapy, antitumor and anti-HIV therapy [14].

Various lignans exert antitumor, antimitotic and antiviral activity and inhibit specific enzymes [15]. Lignans strongly suppressed the incorporation of [^{3}H]thymidine, [^{3}H]uridine, and [^{3}H]leucine into HL-60 cells, indicating cessation of DNA, RNA and/or protein synthesis of leukemic cells [16].

Saponins are steroid or triterpenoid glycosides which derive their name from their ability to form stable, soap-like foams in aqueous solutions and several biological effects which include immunostimulant and antitumourigenic properties [17]. Saponins have also been shown to be anti-inflammatory [18]. In the present study, TLC and staining with anisyl aldehyde in sulfuric acid was used to ascertain its presence.

Various studies by Kuznetzova et al., Rao and Sung, Konoshima et al., Marino et al., Mimaki et al. and Podolak et al. have shown that saponin isolates specifically inhibit the growth of cancer cells *in vitro* [19-25]. Triterpenoid saponins (avicins from *Acacia victoriae*) selectively inhibited growth of tumor cell lines by cell cycle arrest and induction of apoptosis (Mujoo et al.) and reduced both tumor incidence and multiplicity in a murine skin carcinogenesis model [26].

Cytotoxicity, analgesic, antispasmodic and antibacterial are the main activities that have been associated with alkaloids [27]. The biogenesis principle of amino acid (biogenetic amine) and non-amine component represents the common basis of all alkaloids. The classification of alkaloids is based on the heterocycle's structure or on the biogenetic origin. Majority of this compound are derived from the amino acids lysine, tryptophan, tyrosine and arginine (Wagner and Bladt). *Catharanthus roseus* (L.) G. Don (also known as Madagascar periwinkle) leaves contain of two indole alkaloids, vinblastine and vincristine, both of which are well-established in medical oncology since decades [28]. *Vinca* alkaloids kill cancer cells by inhibiting tubulin polymerization during mitotic spindle formation [29].

Cardioactive glycosides are known to lower the blood pressure [30]. A group of glycosidic plant ingredients is called cardioactive steroid glycosides and cause specific myocardial effects owing to special structural characteristics. Cardiac glycosides (CGs) are natural compounds that are potent inhibitors of the plasma membrane Na^+/K^+-ATPase leading indirectly to the intracellular accumulation of Ca^{2+} ions which can lead to myocardial contractility. CGs tends to exert potent antineoplastic effects by increasing the immunogenicity of dying cancer cells [31]. Digitoxigenin can be classified as a cardioactive steroid aglycone's prototype. Especially the *cis-trans-cis* connection of the steroid skeletal structure's rings A-B-C, which is derived from the pregnan's C21-frame (hydroxylated at C3 and C14, wearing an unsaturated lactone ring at C17), causes the cardiac effects (Wagner and Bladt). Digitalis has been shown to block cell proliferation and in non-toxic concentrations has been shown to induce apoptosis in different malignant cell lines [32].

Anthracene derivatives are the characteristic ingredients of hydroxyanthraquinone drugs. They usually contain C- and O- bound sugar moieties. The sugar's chemical bonding to the aglycone's different C- atoms leads to more variations (Wagner and Bladt). The most abundant anthraquinone of rhubarb, a traditional Chinese medicine, emodin, which has been seen to have the capacity of inhibiting cellular proliferation, apoptosis induction and prevention of metastasis reported to act through tyrosine kinases, phosphoinositol 3-kinase (PI3K), protein kinase C (PKC), nuclear factor-kappa B (NF-κB), and mitogen-activated protein kinase (MAPK) signaling cascades [33].

Valepotriates are triesters of a terpenoid, trihydric alcohol with the structure of an iridoid cyclopenta-c-pyran with an attached epoxide ring (Wagner, 1996). They have been seen to exhibit weak to moderate cytotoxicity against the human metastatic prostate cancer cell line, PC-3M [34].

The results obtained in this study thus suggest that the identified phytochemical compounds may be bioactive constituents against cancer and represent a valuable reservoir of compounds deserving substantial medicinal merit.

Conclusion

The present study shows the presence of medicinally important constituents in medicinal plants of the Kakamega County, Kenya, which could serve as valuable sources for useful drugs. We postulate that the anticancer activity is linked to the various constituents of the plants as cross-referenced with published data. In traditional medicine practice, these plants are strongly recommended and further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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