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IN VITRO ANTIFUNGAL, ANTI-INFLAMMATORY AND CYTOTOXIC ACTIVITIES OF RUMEX ABYSSINICUS RHIZOME EXTRACT AND BIOASSAY-GUIDED ISOLATION OF CYTOTOXIC COMPOUNDS FROM RUMEX ABYSSINICUS

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ABSTRACT. *Rumex abyssinicus* showed strong cytotoxicity against HeLa cells (IC_{50} = 22.25 μg/mL) and weak cytotoxicity against PC3 and BJ cells with percent inhibition of 58.6, 25.8 and 29.7% at 30.0 μg/mL. It showed moderate antifungal activity against *Aspergillus niger* with a percent growth inhibition of 55.5% at 3000 μg/mL. It also strongly inhibited oxidative burst with IC_{50} value of 24.8 μg/mL. DCM (100%) and DCM: EtOAc (1:1) fractions showed strong cytotoxicity against HeLa cells, whilst pet ether: DCM (1:1) fraction showed strong cytotoxicity against PC3 cells with IC_{50} values of 29.3, 26.3 and 24.3 μg/mL, respectively. Moreover, the DCM: EtOAc (1:1) fraction inhibited ROS production with IC_{50} value of 18.8 μg/mL. Cytotoxic fractions afforded chrysophanol (**1**), physicon (**2**), emodin (**3**), citreorosein (**4**) and β-sitosterol (**5**). Among the isolated compounds, emodin (**3**) showed strong cytotoxicity against HeLa cells, whilst chrysphanol (**1**) and physicon (**2**) showed strong cytotoxicity against PC3 cells with IC_{50} values of 8.94, 22.5, and 28.5 μM, respectively. In addition, emodin (**3**) and citreorosein (**4**) showed strong inhibition against ROS production with an IC_{50} value of 16.2 and 38.2 μg/mL. The findings of this study suggest *R. abyssinicus* as a good candidate for cancer and inflammation management.

KEY WORDS: Polygonaceae, *Rumex abyssinicu* Jacq., Cytotoxic, Antifungal, Anti-Inflammatory, Reactive oxygen species

INTRODUCTION

Rumex abyssinicus Jacq. (Polygonaceae), locally named in Amharic "mekmako", is an indigenous perennial herb, up to 3 m tall, with thick and fleshy rhizome. It is a medicinal plant that grows in tropical Africa, including Madagascar, more commonly in cultivated lands. In many countries of tropical Africa, leafs and tender shoots are consumed as vegetable. The rhizomes are used to refine butter and give it a bright yellow color [1]. Furthermore, it is also used as a cosmetic in northern Ethiopia for dying the palms of the hands and feet [2]. Traditional healers use this plant for the treatment of various disorders such as amebiasis, hemorrhoid, hepatitis, common cold, wound, hypertension, toothache, headache, blood pressure, asthma, liver disease, abdominal pain, tuberculosis, lung diseases , leprosy, fever, tumor and cancer [3-6].

The phytochemical studies of *R. abyssinicus* extract revealed the presence of tannins, flavonoids, phenols, quinones, alkaloids, cartenoids, phlobatannins, terpenoids, naphthalenes, stilbenoids, steroid, glycosides, saponins, fats and oils [7-9]. Many compounds are reported from *R. abyssinicus* including chrysophanol (**1**), physcion (**2**), emodin (**3**), citreorosein (**4**), β-sitosterol (**5**), aloe-emodin, emodic acid, chrysophanol-8-β-D-glucoside, emodin-β-8-O-D-glucoside, physcion-β-8-O-D-glucoside, bianthrone, emodin-chrysophanol, helminthosporin, epicatechin,

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epicatechin-3-O-gallate, epicatechin-3-O-(4″-O-methyl)gallate, methyl gallate, oxalic acid, phenolic acid, rumexamide, oleanolic acid, lupeol, 3β,28-dihydroxylup-20(22)-ene 3β-dihydroxylup-20(29)-en-28-oic acid, stigmasterol, stigmasterol-3-O-β-D-glucoside stigmastane-3,6-dione and ergosta-6,22-diene-3,5,8-triol [2, 10-13].

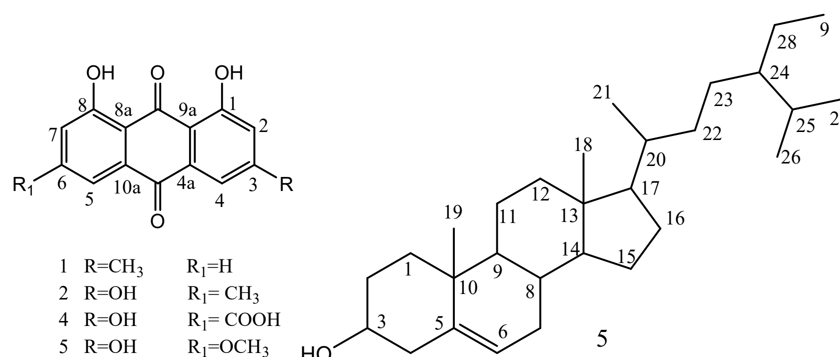

Figure 1. Compounds isolated from cytotoxic fractions of *R. abyssinicus*.

R. abyssinicus reported to demonstrate wide range of bioactivities including antibacterial [14], antiviral [14], cholinesterase inhibitor [11], anthelmintic [15], wound healing [16], anti-inflammatory [14, 16], antioxidant [17], lytic activities against zoospores, *Trypanosomacidal* and anti-tumor [19]. In addition, the *R. abyssinicus* showed a chemopreventive potential against dimethylhydrazine-induced colon carcinogenesis in rats, and it also displayed anticancer activity in prostate, brain, breast, and leukemia cell cultures [6]. The current study was conducted to evaluate the antifungal, anti-inflammatory and cytotoxic activities of *R. abyssinicus* rhizome extract and to isolate lead compounds from cytotoxic fractions.

RESULTS AND DISCUSSION

Cytotoxic activity

The 80% EtOH extract of *R. abyssinicus* exhibited cytotoxicity against cervical cancer (HeLa) and prostate cancer (PC3) cell lines. *R. abyssinicus* inhibited the proliferation of HeLa and PC3 cancer cells by 58.6% and 25.8% at 30 μg/mL. However, the extract exerted less toxicity to normal cells (BJ) with a percent inhibition of 29.7% at 30 μg/mL. *R. abyssinicus* displayed potent cytotoxicity against HeLa cells with an IC_{50} value of 22.25 ± 0.7 μg/mL, but lower compared to standard drug doxorubicin (IC_{50} = 0.9 ± 0.14 μg/mL). In agreement with the current findings, Girma et al. (2013) reported that the 80% methanol in water rhizome extract of *R. abyssinicus* showed a chemopreventive potential against dimethylhydrazine induced colon carcinogenesis in rats, and suggested COX-2 inhibition by the anthraquinones in the extract could be one mechanism for the observed chemopreventive effect [6].

The extract of *R. abyssinicus* was further partitioned sequentially into eight fractions using different polarities (Table 1), in order to determine the characteristics of cytotoxic phytochemicals present in them. Due to solubility problems in DMSO, cytotoxic activity of petroleum ether (100%) eluted fraction was not conducted. Among the fractions tested, 100% DCM (IC_{50} = 24.3 ± 1.4 μg/mL) and 1:1 of DCM: EtOAc (IC_{50} = 29.3 ± 5.5 μg/mL) eluted fractions displayed strong cytotoxicity against cervical cancer HeLa cells and suppressed the proliferation of HeLa cells by 65.2% and 56.2% at 30 μg/mL, respectively. Similarly, the pet ether: DCM (1:1) eluted fraction

showed significant cytotoxicity against PC3 cells with IC_{50} value 26.3 ± 1.2 µg/mL and inhibited the proliferation of PC3 cells by 64.9% at 30 µg/mL.

Table 1. Cytotoxic activity of *R. abyssinicus* extract, fractions and standard drugs.

Crude extract, fractions and standard drug	% inhibition on HeLa		% inhibition on PC3		% inhibition on BJ	
	30 µg/mL	IC_{50} ± SD	50 µg/mL	IC_{50} ± SD	50 µg/mL	IC_{50} ± SD
Crude extract	58.6	22.8 ± 0.3	25.8		29.7	
Pet ether	INS		INS		INS	
Pet ether: DCM (1:1)	9.14		64.9	26.3± 1.2	16.6	
DCM	65.2	24.3 ± 1.4	33.3		29.59	
DCM: EtOAc (1:1)	56.2	29.3 ± 5.5	38.2		54.7	44.4 ± 2.7
EtOAc	14.5		38.9		2.83	
EtOAc : MeOH (1:1)	35.6		1.03		-0.7	
MeOH	-9.5		-50.3		9.6	
MeOH : H_2O (1:1)	4.67		2.74		4.2	
Doxorubicin	101.2	0.9 ± 0.14	89.9		1.9 ± 0.08	
Cycloheximide					89.9	0.8 ± 0.1

DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, H_2O = water, Pet ether = petroleum ether.

The results in Table 1 indicated that the pet ether: DCM (1:1) and DCM (100%) fractions showed different cytotoxicity against HeLa and PC3 cells. Pet ether:DCM (1:1) fraction was found to be significantly cytotoxic to PC3 cells (IC_{50} = 26.3 ± 1.2 µg/mL, 64.9% inhibition at 30 µg/mL) but very weakly cytotoxic to HeLa cells (9.1% inhibition at 30 µg/mL). This finding indicated the responsible compounds/dose-response against the two cancer cells were different. On the other hand, DCM (100%) and DCM:EtOAc (1:1) eluted fractions were demonstrated to have comparable toxicity against HeLa and PC3 cancer cells. This may be due to the responsible compounds/dose-response of fractions being the same or exerting similar toxicity to HeLa and PC3 cancer cells. The presence of the same compounds in different fractions was also observed, but their quantity might be increased or decreased depending on the polarity of the compound and the eluent solvent used. An increment in percent inhibition of proliferation of cancer cells and a decrease in IC_{50} value of eluents indicated the cytotoxic activity of *R. abyssinicus* was related to responsible bioactive compounds and not to synergistic effect of chemical constituents found in crude extract. Furthermore, the secondary metabolites eluted from the column by less polar solvents were rich in steroids and anthraquinones, which are known to possess cytotoxic effects on cancer cells.

Anti-inflammatory activity

ROS are produced as by-products of normal biochemical reactions in the human body [20]. In inflammatory conditions, NADPH oxidases residing in the immune cells are activated and generate ROS in large quantities, creating an oxidative burst. Overproduction of ROS deregulates the cellular functions, which in turn enhances the inflammatory condition. Therefore, inhibition of ROS-induced oxidative burst is a potential therapeutic to prevent and manage inflammatory-mediated diseases. The plant extract that suppresses the production of ROS may be of paramount importance in regulating diseases that originate from immune cell disturbances. The effect of *R. abyssinicus* extract, fractions, or compounds on production of intracellular ROS from serum opsonized zymosan activated whole blood phagocytes was evaluated by luminol enhanced chemiluminescence technique. In this assay, luminol is used as a probe having a low molecular weight. It goes inside the cell and detects intracellular ROS.

The extract of *R. abyssinicus* significantly inhibited the production of whole blood ROS by 75.5% at 50 µg/mL with IC_{50} value of 24.8 ± 2.59 µg/mL. However, the inhibitory effect of extract on production of ROS from whole blood was lower compared to the standard drug ibuprofen with an IC_{50} value of 11.2 ± 1.9 µg/mL. In agreement with the current findings, Mulisa *et al.* [16] reported a dose-related anti-inflammatory activity of 80% methanol extract of the rhizomes of *R. abyssinicus* on carrageenan-induced mice paw edema following oral administration and attested the activity to secondary metabolites which have anti-inflammatory activity including tannins, flavonoids, steroids and anthraquinones [16].

Table 2. ROS production inhibition of *R. abyssinicus* extract and fractions and standard drugs.

Crude extract , fractions and standard drug	% inhibition of ROS	
	50 µg/mL	IC_{50} ± SD (µg/mL)
Crude extract	75.5	24.8 ± 2.59
Pet ether	30.5	
Pet ether:DCM (1:1)	30.5	
DCM	58.8	39.8 ± 7.2
DCM:EtOAc (1:1)	89.8	18.8 ± 0.9
EtOAc	53.1	44.1 ± 2.3
EtOAc:MeOH (1:1)	79.4	26.3 ± 0.7
MeOH	22.7	-
MeOH:H_2O (1:1)	15.1	-
Ibuprofen	73.2*	11.2 ± 1.9

DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, H_2O = water, Pet ether = petroleum ether, *= the % production of ROS inhibition was done at a concentration of 25 µg/mL.

This inhibition of whole blood ROS production by the crude extracts could be due to specific phytochemicals present in them. Therefore, the crude extract of *R. abyssinicus* was further partitioned sequentially into eight solvents having different polarities (Table 2), and the capability of preventing the formation of ROS of each fraction was tested. Among the tested fractions, DCM: EtOAc (1:1), EtOAc:MeOH (1:1), DCM (100%), and EtOAc (100%) fractions inhibited more than 50% production of intracellular ROS from zymosan activated whole blood phagocytes by 89.8%, 79.4%, 58.8% and 53.1% at a concentration of 50.0 µg/mL, respectively. The DCM: EtOAc (1:1), and EtOAc:MeOH (1:1) fractions with IC_{50} values of 18.8 ± 0.9, and 26.3 ± 0.7 µg/mL were found to be strong inhibiters of ROS from human whole blood cells compared to DCM (100%) and EtOAc (100%) fractions with an IC_{50} value of 39.8 ± 7.2 µg/mL, and 44.1 ± 2.3 µg/mL, respectively. However, the standard drug Ibuprofen (IC_{50} = 11.2 ± 1.9 µg/mL) demonstrated stronger inhibitory potential on the production of ROS compared to all fractions.

Antifungal activity

The crude extract was evaluated for antifungal activity against six fungal strains; *Trichophyton rubrum*, *Candida albicans*, *Aspergillus niger*, *Microsporum canis*, *Fusarium lini, Candida glabarata*, and *A. furrigatol* (Table 3). The disk diffusion method was used and the zones of growth inhibition of 80% ethanolic *R. abyssinicus* extract was measured in millimeters (mm) compared to the standard positive control, amphotericin B for *Aspergillus niger* and miconazole for the rest of the tested fungi, and a negative control, DMSO. The zone of inhibition measured was presented in Table 3.

R. abyssinicus extracts demonstrated moderate antifungal activity against *Aspergillus niger* with growth inhibition of 55.5% at a concentration of 3 mg/mL. It also showed low antifungal activity against *Trichophyton rubrum*, *Microsporum canis*, and *Fusarium lini* pathogenic fungal

strains *Aspergillus niger* is a fungal strain that causes a disease called "black mold" on certain fruits, produce potent mycotoxins; and is also a cause of pathogenic allergens generally associated with lung infections in individuals with weak immune system [20]. The current findings are consistent with previous study which reported that MeOH, EtOAc and n-BuOH extracts of *R. abyssinicus* can be a potential source of antifungal agents [12].

Table 3. *In vitro* antifungal activity of *R. abyssinicus* extracts against pathogenic fungal strains.

Fungal strain	% growth inhibition of (3mg/mL)	% growth inhibition of standard drug	
		Amphotericin B	Miconazole
Trichophyton rubrum	20.4	-	70.0
Candida albicans	NI	-	110.0
Aspergillus niger	55.5	20	-
Microsporum canis	30.0	-	95.4
Fusarium lini	25.2	-	73.25
Candida glabarata	NI	-	110.8
A. furrigatol	NI	-	100.0

NI = no inhibition.

Isolation of compounds from cytotoxic fractions

Five compounds were isolated from the cytotoxic fractions of *R. abyssinicus*. The spectroscopic data of isolated compounds agree well with those reported for chrysophanol (**1**) [21], physcion (**2**) [22], emodin (**3**) [23], citreorosein (**4**) [24] and β-sitosterol (**5**) [25].

Cytotoxic activity of compounds

The isolated constituents were estimated for their cytotoxic effects against human cervical cancer (HeLa) and human prostate cancer (PC3) cells. Among the tested compounds, chrysphanol (**1**) and physicon (**2**) and emodin (**3**) demonstrated strong cytotoxic activity against HeLa/PC3 cells with an IC50 values < 30 μM. According to the American National Cancer Institute (NCI), the compound is said to have cytotoxic activity if the IC_{50} value is < 30 μg/mL [26].

Emodin (**3**) inhibited the growth of HeLa and PC3 cancer cells by 76.7 and 36.6% at 30 μM. It showed strong cytotoxicity to HeLa cells with an IC_{50} value of 8.94 ± 1.12 μM. Deng *et al.* [26] reported that emodin (**3**) demonstrated a dos-dependent cytotoxic effect against PC3 cells [27]. Similarly, Yuenyongsawad *et al.* [27] reported that emodin (**3**) demonstrated cytotoxic effect against HeLa cells with an IC_{50} value of 0.86 μg/mL [27]. Emodin (**3**) is also known for its cytotoxic effect against many human cancer cells such as prostate cancer (PC3), liver cancer, lung adenocarcinoma, lung squamous carcinoma, promyelo leukemia, cervical cancer, non-small cell lung cancer, ovary cancer, melanoma cancer, colon cancer, hepatic carcinoma, breast cancer, chronic myeloid leukemia, and gastric cancer cells through its effects across multiple signaling pathways [26-30].

Chrysphanol (**1**) and physicon (**2**) demonstrated better cytotoxicity to PC3 cells with IC_{50} values of 22.5 ± 1.7 and 28.5 ± 2.2 μM. They exhibited weak cytotoxicity to HeLa cells. Chrysphanol (**1**) and physicon (**2**) reported to exhibit growth inhibitory activity against HeLa cells in a dose dependent manner [31]. According to Lee *et al.* [30], chrysphanol (**1**) and physicon (**2**) showed strong cytotoxic effects against several cancer cell lines, including non-small cell lung, ovary cancer, melanoma cancer, central nervous system, colon cancer, breast cancer, and human colorectal adenocarcinoma cells [27, 28].

Citreorosein (**4**) showed notable cytotoxicity to HeLa and PC3 cells and inhibited the growth by 48.3 and 34.5% at 30 μM. Lu *et al.* [32] reported that citreorosein (**4**) suppresses the gene expression of pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin

(IL)-6 and IL-1β in mouse bone marrow-derived mast cells (BMMCs) stimulated with phorbol 12-myristate 13-acetate (PMA) plus the calcium ionophore [32].

Anthraquinone-based compounds play a significant role in treatment of cancers by chemotherapeutics agents. They used as a core chemical template to achieve structural modifications, resulting in the development of new anthraquinone based compounds as promising anticancer agents. Some of the anthraquinone scaffold containing drugs such as doxorubicin, epirubicin, valrubicin, pixantrone, and mitoxantrone are currently in clinical use for various types of cancer treatments. Mechanistically, most of the anthraquinone-based compounds inhibit cancer progression by targeting essential cellular proteins [33].

β-Sitosterol (**5**) also demonstrated weak cytotoxic activity against prostate cancer PC3 cell lines. β-sitosterol (**5**) reported to exhibit a cytotoxic effect against HeLa cells [34]. β-Sitosterol (**5**) reported to exhibit anticancer properties against breast, prostate, colon, pancreatic, lung, stomach, and ovarian cancers by interfering with multiple cell signaling pathways including cell cycle and apoptosis [34-36]. Numerous studies have evidenced that the anticancer effect of β-sitosterol (**5**) is related to the induction of apoptosis through blockade of multiple cell signalling mechanisms [35].

Anti-inflammatory activity of compounds

The inhibitory potential of compounds on the production of intracellular ROS from serum opsonized zymosan activated whole blood phagocytes is depicted in Table 4. The data collected revealed that among all tested compounds; emodin (**3**) and citreorosein (**4**) significantly inhibited the production of ROS with IC_{50} values of 16.20 ± 0.9 and 24.30 ± 1.6 μg/mL, respectively. However, the ROS production inhibition potential of emodin (**3**) and citreorosein (**4**) was lower compared to the standard drug Ibuprofen (IC_{50} value of 11.20 ± 1.9 μg/mL). Hang *et al.* [37] reported that the structure-activity relationship of emodin (**3**) indicates that the free hydroxyl group at position 3 of the anthraquinone nucleus plays an important role in the immunosuppressive effect [37].

Chrysophanol (**1**), physcion (**2**) and β-sitosterol (**5**) activated the production of intracellular ROS and increased the ROS production by 9.6, 10.1, and 22.7%. Literature report indicated a significant increase in intracellular ROS in chrysophanol (**1**), physicon (**2**), β-sitosterol (**5**) treated cells when compared to the control cells [38-40]**.** ROS has a crucial role in cell signaling and cellular functions. Mounting evidences suggest that an abnormal increase of ROS is often observed in cancer cells and that this biochemical feature can be exploited for selective killing of malignant cells. Although high levels of ROS contribute to carcinogenesis and other diseases related to oxidative damage, appropriate levels of ROS have been shown to be indispensable for cell survival, apoptosis, and differentiation [41].

Table 4. Cytotoxicity and ROS inhibition activity of compounds isolated from cytotoxic fractions.

Compounds	% inhibition on HeLa		% inhibition on PC3		% inhibition of ROS	
	30 μM	IC_{50} ± SD	30 μM	IC_{50} ± SD	50 μg/mL	IC_{50} ± SD
Chrysophanol (**1**)	33.8 %	-	64.5	22.5 ± 1.7	-9.6	-
Physcion (**2**)	29.5	-	56	28.5 ± 2.2	-10.1	-
Emodin (**3**)	86.7	8.94 ± 1.12	36.6	-	74.2	16.2 ± 0.9
Citreorosein (**4**)	48.3	-	34.5	-	56.6	38.2 ± 2.4
β-sitosterol (**5**)	8.4	-	16.5	-	-22.7	-

Extracts, fractions, or compounds that have a potential to inhibit ROS-induced oxidative burst, can serve as effective anti-inflammatory agents. Therefore, these results provided evidence for the anti-inflammatory potential of *R. abyssinicus* extract, fractions, and isolated active

compounds. Furthermore, it indicates the potential application of *R. abyssinicus* in the prevention and management of ROS-induced inflammatory conditions as a natural source of anti-inflammatory drug candidates.

EXPERIMENTAL

Materials

All solvents were ACS grade (Carlo Erba reagents S.A.S, Val de Reuil, France); Dulbecco's Modified Eagle Medium (Gibco, ThermoFisher Scientific, USA); fetal bovine serum (Thermo-Scientific and ScienCell, USA); penicillin-streptomycin (Invitrogen, ThermoFisher Scientific, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Invitrogen, ThermoFisher Scientific, ScienCell, USA); Sabouraud dextrose agar (Thermo-Scientific and ScienCell, USA); amphotericin B (Formepharma, Pakistan), Miconazole (Formepharma, Pakistan); doxorubicin (ICN, USA); Hanks Balanced Salt Solution ($HBSS^{++}$) (Sigma, St. Louis, USA); zymosan (Fluka, Buchs, Switzerland); Luminol (Research Organics, Cleveland, USA); 96-well white half area plates (Costar, NY, USA). A human cervical cancer (HeLa), human prostate cancer (PC3), and normal human foreskin fibroblasts (BJ) cells were obtained from the cell bank at Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International center for chemical and biological sciences (ICCBS), University of Karachi, Karachi, Pakistan.

Apparatus and instrument

The compounds reported in this work were isolated using vacuum liquid chromatography (VLC) which carries 1 kg silica gel 60HF, and three sizes (big, medium, and small) column chromatography (CC) which can carry 200.0, 80.0, and 50.0 g silica gel 60HF, respectively. Fractions collected from VLC were purified using column (50mm x50cm) which can carry 200 g silica gel 60HF. TLC was performed on pre-coated plates (Silica gel 60 F254, 230-400 mesh, Merck) and Al_2O_3 plates. Spots were detected by observation under UV light (Vilber Lourmat). Spraying agents used were 10% cerium (IV) sulphate ($Ce(SO_4)_2$) and 5% KOH. Melting point (mp) was determined in capillary tube with a digital electrothermal melting point apparatus. UV-Vis spectral measurements were done on a Shimadzu UV-VIS recording spectrophotometer, UV-160, spectronic genesys spectrophotometer. The IR (KBr) spectral measurements were done on a Perkin Elmer 1600 and Pye Unicam Infrared spectrophotometer SP3-300. Electron ionized mass spectrometry (EI-MS) was carried out using JEOL-600H-1. The nuclear magnetic resonance (NMR) spectra were recorded in deuterated solvents ($CDCl_3$ or $(CD_3)_2CO$) on a Bruker Avance III 400 and Avance Neo 600 MHz NMR spectrometers. All chemical shifts (δ) are reported in parts per million (ppm) with the solvent signal as a reference relative to TMS ($\delta = 0$) as internal standard, while the coupling constants (J) are given in Hertz (Hz).

Plant collection

The rhizome of *R. abyssinicus* was collected in October, 2018, inside Arat Kilo Campus, Addis Ababa University, Addis Ababa, Ethiopia. This plant was identified and authenticated by a taxonomist and voucher specimen (JA-04-2019) was deposited at the National Herbarium, Department of Biology, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia. Botanical names have been transcribed according to the nomenclature system used by The World Flora Online (http: //www.theplantlist. org).

Extraction and fractionation

The air-dried and pulverized rhizome of *R. abyssinicus* (1.0 kg) was soaked in EtOH:H_2O (8:2) (5 L) for two weeks at room temperature (20-25 °C). The combined extracts were filtered with Whatman filter paper (type 2) and concentrated under pressure using rotary evaporator (Heidolph Hei-VAP, Germany) preset at 40 ºC yielding a dark red powder (41.0 g). The extract (40.0 g) was pre-adsorbed on silica gel 60HF (60.0 g) and introduced to vacuum liquid chromatography (VLC) packed with silica gel 60HF (500 g) as stationary phase. The elution was carried out with different solvent gradient systems using (2000 mL each): pet ether (100%), pet ether: DCM (1:1), DCM (100%), DCM: EtOAc (1:1), EtOAc (100%), EtOAc: MeOH (1:1), MeOH (100%), and MeOH: H_2O (1:1) successively. The eluents were separately collected and evaporated to dryness to afford 3.5, 7.6, 9.6, 0.9, 4.5, 2.6 g 1.9, and 1.0 g, respectively.

Isolation of compounds

Pet ether: DCM (1:1), DCM (100%), and DCM: EtOAc (1:1) eluted fractions were separately pre-adsorbed on silica gel 60HF and fractionated on different size column packed with silica gel 60HF. A pet. ether: DCM (1:1) fraction (7.0 g) was pre-adsorbed on silica gel 60HF (10.0 g), packed with silica gel 60HF (180 g) on CC (50 mm x 50 cm), and eluted with pet ether: acetone (99.75:0.25, 99.5:0.5, 99:1, 49:1, 24:1(2), 9.5:0.5, 9:1, 8:2, 7:3, 1:1 400 mL each), successively. A total of 40 fractions (100 mL each) were collected (F1–F40). Fraction F9-F14 (1.0 g) was adsorbed on silica gel 60HF (1.0 g), packed with silica 60HF (50.0 g) on CC (25 mm x 50 cm), and eluted with pet ether: acetone (99.5: 0.5, 99:1, 49:1(2), 24:1(2), 9:1,8:2 200 mL each). Out of 40 fractions (50 mL each) collected (Fa1-Fa40), fraction Fa21-Fa23 afforded compound **1** (250 mg) as a clear orange solid after solvent removal under reduced pressure. In addition, fraction F24-F30(1.9 g) was also pre-adsorbed on silica gel 60HF (2.0 g), packed with silica gel 60HF (50.0 g) on CC (25 mm x 50 cm), and eluted with pet ether: acetone (99:1, 49:1, 24:1(2), 9:1, 8:2, 1:1 200 mL each) to give Fb1-Fb56 a fractions (25 mL each). Compound **2** (27.5 mg) was obtained from Fb18-Fb21 fraction as an orange solid.

DCM (100%) fraction (9.0 g) was adsorbed on silica gel 60HF (9.0 g), packed with silica gel 60HF (200.0 g) on CC (50 mm x 50 cm), and eluted with pet ether: acetone (99.75:0.25, 99.5:0.5, 99:1, 49:1, 24:1, 9.5:0.5, 9:1, 8:2, 7:3, 1:1 400 mL each) successively. A total of 40 fractions (100 mL each) were collected (F1–F40). Fraction F12-F18 (1.0 g) pre-adsorbed on silica gel 60HF (2.0 g), packed with silica gel 60HF (50.0 g) on CC (25 mm x 50 cm) and eluted with pet ether: acetone (99.75:0.25, 99.5: 0.5, 99:1, 49:1, 24:1, 9:1, and 8:2 200 mL each). A total of 30 fractions (50 mL each) were collected (Fc1-Fc30), and fraction Fc10-Fc14 and Fc18-Fc22 afforded compounds **1** (12.2 mg) and compound **2** (15.6 mg), respectively, as orange solids. In addition, fraction F22-F29 (1.7 g) was pre-adsorbed on silica gel 60HF (2.0 g) and packed with silica gel 60HF (50.0 g) on CC (25 mm x 50 cm) and eluted with pet ether: acetone (99.75:0.25, 99.5: 0.5, 99:1, 49:1, 24:1, 9.5:0.5, 9:1, 8:2, 7:3, and 1:1 200 mL each). A total of 80 fraction (25 mL each) were collected (Fd1-Fd80). Fraction Fd32-Fd37 and Fd59-Fd65 afforded compound **24** (725 mg) and compound **4** (140 mg) as white and red solids, respectively.

DCM: EtOAc (1:1) fraction (0.7 g) was pre-adsorbed on silica gel 60F(1.0 g), packed with silica gel 60HF (50.0 g) on CC (25 mm x 50 cm), and eluted with pet ether:acetone (49:1, 24:1, 9.5:0.5, 9:1 (2), 8:2 (2), 7:3, 1:1, and 0:1 200 mL each) successively. A total of 40 fractions (25.0 mL each) were collected (Fe1–Fe80). Compound **4** (17.1 mg) and compound **5** (27.8 mg) were obtained as a red and orange solids from fraction Fe32-Fe34 and Fe44-Fe56, which were eluted with 9:1 and 8:2 pet ether in acetone.

Characterization and identification of compounds

Chrysophanol (1). An orange-red powder; mp 194-197 ºC; UV-Vis (MeOH) λ_{max} nm: 259, 290 and 433; IR (KBr): ν_{max} cm^{-1}: 3417, 1676, and 1631; EI MS: $[M]^+$ peak at m/z 254.0 corresponding

$C_{15}H_{10}O_4$ (calc. mass, 254.0579); ^{1}H-NMR (500 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant J in Hz: δ_H 1.43 12.11 (1H, *s*, OH-8), 12.00 (1H, *s,* OH-1), 7.81 (1H, *dd*, J = 7.55, 0.9 , H-5), 7.65 (1H, *t*, J = 8.45, H-6), 7.64 (1H, *d*, = 0.9 Hz, H-4), 7.27 (1H, *dd*, J = 8.45, 0.9, H-7), 7.09 (1H, *br.s*, H-2), 2.45 (3H, *s*, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 162.8 (C-1), 124.4 (C-2), 149.4 (C-3), 121.4 (C-4), 119.9 (C-5), 137.0 (C-6), 124.6 (C-7), 162.5 (C-8), 192.6 (C-9), 182.0 (C-10), 22.3 (C-11), 133.3 (4a), 115.9 (8a), 113.8 (9a), 133.7 (10a).

*Physicon (**2**)*. An orange-yellow powder; mp 204-208 ºC; UV-Vis (MeOH) λ_{max} nm: 265, 289 and 439; IR (KBr) ν_{max} cm^{-1}: 3468, 1672, and 1629; EI MS: $[M]^+$ peak at m/z = 284.1 corresponding to molecular formula (calc. mass, 284.0685); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant J in Hz: δ_H 12.35 (1H, *s*, OH-8), 12.15 (1H, *s*, OH-1), 7.65 (1H, *s*, H-4), 7.39 (1H, *d*, J = 2.5, H-5), 7.10 (1H, *s*, H-2), 6.71 (1H, *d*, J = 2.5, H-7), 3.96 (3H, *s*, OCH_3), 2.47 (3H, *s*, H-11); ^{13}C NMR (100 MHz, $CDCl_3$): δ_H 165.2 (C-1), 124.5 (C-2), 148.5 (C-3), 121.3 (C-4), 106.8 (C-5), 166.6 (C-6), 108.2 (C-7), 162.5 (C-8), 190.8 (C-9), 182.1 (C-10), 22.2 (C-11), 133.2 (4a), 110.3 (8a), 113.7 (9a), 135.3 (10a), 56.1 (OCH_3).

*Emodin (**3**)*. Pinkish orange needles; mp 254-256 °C; EI MS: a $[M]^+$ peak at m/z = 270.1 corresponding to molecular formula $C_{15}H_{10}O_5$ (calc. mass, 270.0528); UV-Vis (MeOH) λ_{max} nm: 265, 289 and 437; IR (KBr) ν_{max} cm^{-1}: 3388, 2923,1687, 1633; ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant J in Hz: δ_H 7.56 (1H, *s*, H-4), 7.13 (1H, *s*, H-2), 7.24 (1H, *d*, J = 2.24, H-5), 6.65 (1H, *d*, J = 2.28, H-7) and δ 2.46 (3H, *s*, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 163.2 (C-1), 124.9 (C-2), 149.5 (C-3), 121.5 (C-4), 109.7 (C-5), 166.5 (C-6), 108.8 (C-7), 166.2 (C-8), 191.7 (C-9), 182.2 (C-10), 134.2 (4a), 110.4 (8a), 114.4 (9a), 136.6 (10a), 21.9 (CH_3).

*Citreorosein (**4**)*. An orange amorphous powder; mp 284-288 °C; EI MS: $[M]^+$ peak at m/z = 286.1 corresponding to molecular formula $C_{15}H_{10}O_6$ (calc. mass, 286.0477); UV-Vis (MeOH) λ_{max} nm: 267, 289, 442; IR(KBr) ν_{max} cm^{-1}: 3402, 2923, 1634; ^{1}H-NMR (500 MHz, C_3D_6O), chemical shift δ in ppm, coupling constant J in Hz: δ_H 7.75 (1H, *d*, J = 0.4, H-4), 7.31 (1H, *brs*, H-2), 7.26 (1H, *d*, J = 2.0, H-5), 6.66 (1H, *d*, J = 2.0, H-7), 4.76 (2H, *s*, H-11); ^{13}C NMR (125 MHz, C_3D_6O): δ_C 165.2 (C-1), 121.7 (C-2), 153.9 (C-3), 118.1 (C-4), 109.8 (C-5), 166.3 (C-6), 108.8 (C-7), 166.7 (C-8), 191.7 (C-9), 182.2 (C-10), 134.4 (4a), 110.4 (8a), 115.2 (9a), 136.7 (10a), 63.6 (C-11).

*β-Sitosterol (**5**)*. White solid; mp 134-136 ºC; UV-Vis ($CHCl_3$) λ_{max} nm: no absorption 600-200; IR (KBr) ν_{max} cm^{-1}: 3415, 2937, 2843, 1659, 1447, 1381 and 1048; EI MS: $[M]^+$ peak at m/z 414.4 corresponding to molecular formula $C_{29}H_{50}O$ (calc. mass, 414.3862); ^{1}H-NMR (500 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant J in Hz: δ 5.33 (1H, *m,*, H-6), 3.50 (1H, *m*, H-3), 0.99 (3H, *s*, H-19), 0.90 (3H, *d,* J = 6.55, H-21), 0.83 (3H, *t*, J = 7.45, H-29), 0.81 (3H, *d*, J = 6.85, H-26), 0.79 (3H, *d*, J = 6.8, H-27), 0.66 (3H, *s*, H-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9(C-8), 50.1 (C-9), 36.5 (C-10), 21.1(C-11), 39.8 (C-12), 42.3 (C- 13), 56.8 (C-14), 26.1 (C-15), 28.2 (C-16), 56.1 (C-17), 11.9 (C-18), 19.4 (C-19), 36.1 (C-20), 18.8 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C- 26), 19.0 (C-27), 23.1 (C-28), 12.0 (C-29).

Biological activities

Cell lines and culture conditions

The human cervical cancer (HeLa), prostate cancer (PC3), human fibroblast (BJ) normal cells were separately cultured in Dulbecco's Modified Eagle Medium (DMEA), supplemented with 5% for HeLa and PC3 cells and 10% for BJ cells of fetal bovine serum (FBS), 100 IU/mL of penicillin, 100 µg/mL of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37 ºC.

Cytotoxic activity

The cytotoxicity assays (cell viability test) on HeLa, PC3, and BJ was performed according to microculture MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method as mentioned by Mosmann [42]. Briefly, 100 µL per well of cell solutions (6×10^4 cells per mL HeLa cells, 1×10^5 cells per mL PC3 cells, or 6×10^4 cells per mL BJ cells), were added into 96-well plate and incubated for 24 h at 37 °C. After overnight incubation, medium was removed and 200 µL of fresh medium was added with extract (30 µg/mL)/fraction (30 µg/mL)/compound (30 µM)/standard (30 µM) for 48 h. After this, 200 µL MTT (0.5 µg/mL) was added to each well and incubated further for 4 h at 37 °C. Then 100 µL DMSO was added to each well to dissolve the formazan crystal. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm (PC and HeLa) or 550 nm (BJ) using a microplate reader (Spectra Max Plus, Molecular Devices, CA, USA). Standard drug doxorubicin as a positive control and DMSO as a negative control was used to find the percent growth inhibition or decrease in viable cells. The IC_{50} value of active compounds (inhibition > 50% at 30 µg/mL) was calculated using the EZ-Fit software. The percent growth inhibition was calculated by using the following formula:

$$\text{\% inhibition} = \frac{100-(\text{Mean of O.D. of test substance} - \text{Mean of O.D. of NC}}{(\text{Mean of O.D. of PC} - \text{Mean of O.D. of NC})} * 100$$

where O.D. is optical density, NC is negative control and PC is positive control.

Selectivity index

High SI value (> 2) of a compound suggests selective toxicity against cancer cells, while a compound with SI value < 2 is considered to give general toxicity which can also cause cytotoxicity in normal cells [43]. Each SI value is calculated using the formula:

$$\text{SI} = \frac{\text{IC}_{50}\text{ normal cell}}{\text{IC}_{50}\text{ cancer cell}}$$

Anti-inflammatory activity test

Luminol-enhanced chemiluminescence assay was performed, as described by Helfand *et. al.* [44] with slight modifications. Briefly, 25 µL of 1:20 diluted whole blood in $HBSS^{++}$ was incubated with 25 µL of test sample (50.0 µg/mL for screening and three different concentrations, 1.0, 10.0, and 100.0 µg/mL for active samples), each in triplicate. Control wells received $HBSS^{++}$ and cells, but no test sample. Test was performed in white half area 96 well plates which were incubated at 37 ºC for 15 min in the thermostat chamber of luminometer (Lab systems, Helsinki, Finland). After incubation, 25 µL of 0.30% serum opsonized zymosan (SOZ) and 25 µL of 7×10^{-5} M of intracellular ROS detecting probe, luminol was added into each well, except blank wells (containing only $HBSS^{++}$). The results were monitored as relative light units (RLU) reading, with peak and total integral values set with repeated scans at 50 s intervals, and 1 s point of measuring time. Standard used for the assay was Ibuprofen. The inhibition percentage (%) for each extract was calculated using the following formula:

$$\text{\% Inhibition of ROS} = \frac{(RLU_{control} - RLU_{sample}) \times 100\%}{RLU_{control}}$$

Antifungal activity

The antifungal activity of extract to pathogenic fungi, i.e. *trichophyton rubrum*, *candida albicans*, *aspergillus niger*, *microsporum canis*, and *candida glabarata*, was determined using agar tube dilution method [45]. The fungal strains were grown in Sabouraud dextrose agar (SDA) which

contained 2% maltose (pH 5.5-5.6), prepared by mixing 32.5 g of SDA in 500.0mL water and steamed to dissolve the contents. Into each screw caps tubes 4 mL media was dispensed. The tubes were autoclaved at 121 °C for 15 min. Tubes were allowed to cool to 50 °C and non-solidified SDA was loaded with 66.6 µL of extracts pipetted from the stock solution (180.0 µg/mL) to give the final concentration of 3000 µg/mL. Then the tubes were allowed to solidify in slanting position at room temperature. Each tube was inoculated with a 4mm diameter piece of fungus removed from a mycelial plugs cut from the edge of seven day old cultures. For non-mycelial growth, an agar surface streak was employed. Media supplemented with DMSO was used as negative control and reference antifungal drug Amphotericin B for *Aspergillus niger* and Miconazole for the remaining tested fungal species were used as a positive control. The tubes that were incubated at 27 °C for 7 days and cultures were examined twice-weekly during incubation. Growth in the extract amended media was determined by measuring linear growth (mm) and growth inhibition calculated with reference to the negative control. The % inhibition from 0-39% considered as low, 40-59% as moderate, and 60-69% as good and above 70 as significantly active. The % inhibition of fungal growth for each extract was calculated using the following formula:

$$\%\ \text{Fungal growth inhibition} = 100\% - \frac{\text{linear growth in test (mm)}}{\text{linear growth in control (mm)}} \times 100\%$$

CONCLUSION

The bioassay-guided fractionation and isolation of compounds from *R. abyssinicus* against cancer cells resulted in isolation of cytotoxic compounds chrysphanol (**1**), physicon (**2**) and emodin (**3**) that may be a potential therapeutic agent in treatment of various cancers. The strong oxidative burst inhibitory potential of *R. abyssinicus* extract, fractions and isolated compounds emodin (**3)** and citreorosein (**4**) proved the effectiveness of *R. abyssinicus* and its isolates as natural alternatives to regulate various forms of pro-oxidative, immune disorders and inflammation. The present finding supports the folk medicinal value of *R. abyssinicus* in treatment of different aliments and provides scientific backing for utilizing them in such herbal preparations.

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REFERENCES

1. Edwards, S.; Tadesse, M.; Demissew, S.; Hedberg, I. *Flora of Ethiopia and Eritrea, Volume 2, Part 1: Magnoliaceae to Flacourtiaceae*, Addis Ababa, Ethiopia and Uppsala, Sweden: The National Herbarium, Addis Ababa University; **2000**; p 339.
2. Zelalem, G.A.; Dula, D.E. Isolation, characterization and antibacterial activities evaluation of *Rumex abyssinicus* rootbark extracts. *Nat. Prod. Chem. Res.* **2019**, 7, 1–8.
3. Bussa, N.F.; Belayneh, A. Traditional medicinal plants used to treat cancer, tumors and inflammatory ailments in Harari Region, Eastern Ethiopia. *South African J. Bot.* **2019**, 122, 360–368.
4. Abebe, W. An overview of Ethiopian traditional medicinal plants used for cancer treatment.

Eur. J. Med. Plants **2016**, 14, 1–16.
5. Esubalew, S.T.; Belete, A.; Lulekal, E.; Gabriel, T.; Engidawork, E.; Asres, K. Review of ethnobotanical and ethnopharmacological evidences of some Ethiopian medicinal plants traditionally used for the treatment of cancer. *Ethiop. J. Health Dev.* 2017, 31, 161–187.
6. Girma, B.; Yimer, G.; Makonnen, E. Effect of *Rumex abyssinicus* on preneoplastic lesions in dimethylhydrazine induced colon carcinogenesis in rats. *Complement. Altern. Med.* **2015**, 15(365), 1–7.
7. Awoke, S.; Gedamu, C. Chemical studies and antibacterial activity of the root of *Rumex abyssinicus*. *Earthline J. Chem. Sci.* **2020**, 4, 155–173.
8. Fufa, F.M.; Padmanabhan, R.; Gurmessa, G.T. Phytochemical investigation and in vitro antibacterial evaluation on root extracts of *Rumex abyssinicus*. *Nat. Prod. Chem. Res.* **2016**, 4, 1000239.
9. Tala, M.F.; Ansary, M.W.R.; Talontsi, F.M.; Kowa, T.K.; Tofazzal Islam, M.; Tane, P. Anthraquinones and flavanols isolated from the vegetable herb *Rumex abyssinicus* inhibit motility of phytophthora capsici zoospores. *South African J. Bot.* **2018**, 115, 1–4.
10. Munavu, R.; Mudamba, L.; Ogur, J. Isolation and characterization of the major anthraquinone pigments from *Rumex abysinica*. *Planta Med.* **1984**, 50, 111.
11. Augustin, N.; Nuthakki, V.K.; Abdullaha, M.; Hassan, Q.P.; Gandhi, S.G.; Bharate, S.B. Discovery of helminthosporin, an anthraquinone isolated from *Rumex abyssinicus* Jacq as a dual cholinesterase inhibitor. *ACS Omega* **2020**, 5, 1616–1624.
12. Kengne, I.C.; Donald, L.; Feugap, T.; Njouendou, A.J.; Darille, C.; Ngnokam, J.; Djamalladine, M.D.; Ngnokam, D.; Voutquenne-nazabadioko, L.; Tamokou, J. Antibacterial, antifungal and antioxidant activities of whole plant chemical constituents of *Rumex abyssinicus*. *BMC Complement. Med. Ther.* **2021**, 21(164), 1–14.
13. Tsamo, L.D.F.; Yimgang, L.V.; Wouamba, S.C.N.; Mkounga, P.; Nkengfack, A.E.; Voutquenne-Nazabadioko, L.; Ngnokam, D.; Lenta, B.N.; Sewald, N. A new ceramide (rumexamide) and other chemical constituents from *Rumex abyssinicus* Jacq (polygonaceae): isolation, characterization, antibacterial activities and chemophenetic significance. *Adv. Biol. Chem.* **2021**, 11, 266–282.
14. Getie, M.; Gebre-Mariam, T.; Rietz, R.; Höhne, C.; Huschka, C.; Schmidtke, M.; Abate, A.; Neubert, R.H. Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants *Dodonaea viscosa*, *Rumex nervosus* and *Rumex abyssinicus*. *Fitoterapia* **2003**, 74, 139–143.
15. Peachey, L.E.; Pinchbeck, G.L.; Matthews, J.B.; Burden, F.A.; Mulugeta, G.; Scantlebury, C.E.; Hodgkinson, J.E. An evidence-based approach to the evaluation of ethnoveterinary medicines against strongyle nematodes of equids. *Vet. Parasitol.* **2015**, 210 (1–2), 40–52.
16. Mulisa, E.; Asres, K.; Engidawork, E. Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus J*. (Polygonaceae) in Mice. *BMC Complement. Altern. Med.* **2015**, 15(341), 1–10.
17. Mohammed, S.A.; Panda, R.C.; Madhan, B.; Demessie, B.A. Extraction of bio-active compounds from ethiopian plant material *Rumex Abyssinicus* (mekmeko) root - A study on kinetics, optimization, antioxidant and antibacterial activity. *J. Taiwan Inst. Chem. Eng.* **2017**, 75, 228–239.
18. Worku, N.; Mossie, A.; Stich, A.; Daugschies, A.; Trettner, S.; Hemdan, N.Y.A.; Birkenmeier, G. Evaluation of the in vitro efficacy of *Artemisia annua*, *Rumex abyssinicus*, and *Catha edulis* Forsk extracts in cancer and trypanosoma brucei cells. *ISRN Biochem.* **2013**, 2013, 1–10.
19. Bagchi, D.; Bagchi, M.; Stohs, S.J.; Das, D.K.; Ray, S.D.; Kuszynski, C.A.; Joshi, S.S.; Pruess, H.G. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* **2000**, 148, 187–197.
20. Gautam, A.K.; Sharma, S.; Avasthi, S.; Bhadauria, R. Diversity, pathogenicity and toxicology of *A. niger*: an important spoilage fungi. *Res. J. Microbiol.* **2011**, 6, 270–280.

21. Tutin, B.F.; Williax, H.; Clewer, B. XCIX.— the constituents of rhubarb. *J.Chem. Soc. Trans* **1911**, 99, 946–967.
22. Mellado, M.; Madrid, A.; PeñA-CortéS, H.; LóPez, R.; Jara, C.; Espinoza, L. Antioxidant activity of anthraquinones isolated from leaves *of Muehlenbeckia hastulata* (je. sm.) Johnst. (Polygonaceae). *J. Chil. Chem. Soc.* **2013**, 58, 1767–1770.
23. Jibril, S.; Sirat, H.M.; Basar, N. Bioassay-guided isolation of antioxidants and α-glucosidase inhibitors from the root of *Cassia sieberiana* DC (Fabaceae). *Rec. Nat. Prod.* **2017**, 11, 406–410.
24. Alemu, M.A.; Mekonnen, H.G.; Annisa, M.E. Phytochemical analysis and antibacterial activity on seed and pod extracts of *Crotalaria incana*. *J. Pharm. Pharmacogn. Res.* **2015**, 3, 100–108.
25. Krzyzaniak, L.M.; Antonelli-Ushirobira, T.M.; Panizzon, G.; Sereia, A.L.; Souza, J.R.P.D.; Zequi, J.A.C.; Novello, C.R.; Lopes, G.C.; Medeiros, D.C.D.; Silva, D.B.; Leite-Mello, E.V.D.S. Larvicidal activity against *Aedes aegypti* and chemical characterization of the inflorescences of tagetes patula. *Evid.-based Complement. Altern. Med.* **2017**, 9602368.
26. Ma, X.; Tian, W.; Wu, L.; Cao, X.; Ito, Y. Isolation of quercetin-3-OL-rhamnoside from *Acer truncatum bunge* by high-speed counter-current chromatography. *J. Chromatogr. A.* **2005**, 1070, 211–214.
27. Deng, G.; Ju, X.; Meng, Q.; Yu, Z.J.; Ma, L.B. Emodin inhibits the proliferation of PC3 prostate cancer cells in vitro via the Notch signaling pathway. *Mol. Med. Rep.* **2015**, 12, 4427–4433.
28. Yuenyongsawad, S.; Bunluepuech, K.; Wattanapiromsakul, C.; Tewtrakul, S. Anti-cancer activity of compounds from Cassia garrettiana heartwood. *Songklanakarin J. Sci. Technol.* **2014**, 36, 189–194.
29. Lee, N.J.; Choi, J.H.; Koo, B.S.; Ryu, S.Y.; Han, Y.H.; Lee, S.I.; Lee, D.U. Antimutagenicity and cytotoxicity of the constituents from the aerial parts of *Rumex acetosa*. *Biol. Pharm. Bull.* **2005**, 28, 2158–2161.
30. Hsu, S.C.; Chung, J.G. Anticancer potential of emodin. *BioMedicine* **2012**, 2, 108–116.
31. Akkol, E.K.; Tatlı, I.I.; Karatoprak, G.Ş.; Ağar, O.T.; Yücel, Ç.; Sobarzo-Sánchez, E.; Capasso, R. Is emodin with anticancer effects completely innocent? Two sides of the coin. *Cancers* **2021**, 13, 2733.
32. Ertürk, S.; Özbaş, M.; İmre, S. Anthraquinone pigments from Rumex cristatus. *ACTA Pharmaceutica Sciencia* **2001**, 43, 21-22.
33. Trybus, W.; Król, T.; Trybus, E.; Stachurska, A. Physcion induces potential anticancer effects in cervical cancer cells. *Cells* **2021,** 10, 1–15.
34. Malik, M.S.; Alsantali, R.I.; Jassas, R.S.; Alsimaree, A.A.; Syed, R.; Alsharif, M.A.; Kalpana, K.; Morad, M.; Althagafi, I.I.; Ahmed, S.A. Journey of anthraquinones as anticancer agents– a systematic review of recent literature. *RSC Adv.* **2021**, 11, 35806–35827.
35. Jeong, S.Y.; Zhao, B.T.; Kim, Y.H.; Min, B.S.; Woo, M.H. Cytotoxic and antioxidant compounds isolated from the cork of *Euonymus alatus* Sieb. *Nat. Prod. Sci.* **2013**, 19, 366–371.
36. Bin Sayeed, M.S.; Ameen, S.S. Beta-sitosterol a promising but orphan nutraceutical to fight against cancer. *Nutr. Cancer* **2015**, 67, 1216–1222.
37. Huang, H.C.; Chang, J.H.; Tung, S.F.; Wu, R.T.; Foegh, M.L.; Chu, S.H. Immunosuppressive effect of emodin, a free radical generator. *Eur. J. Pharmacol.* **1992**, 211, 359–364.
38. Lu, C.C.; Yang, J.S.; Huang, A.C.; Hsia, T.C.; Chou, S.T.; Kuo, C.L.; Lu, H.F.; Lee, T.H.; Wood, W.G.; Chung, J.G. Chrysophanol induces necrosis through the production of ros and alteration of ATP levels in J5 human liver cancer cells. *Mol. Nutr. Food Res.* **2010**, 54, 967–976.
39. Trybus, W.; Król, T.; Trybus, E.; Stachurska, A. Physcion induces potential anticancer effects in cervical cancer cells. *Cells* **2021**, 10, 2029.

40. Awad, A.B.; Burr, A.T.; Fink, C.S. Effect of resveratrol and β-sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells. *Prostaglandins Leukot. Essent. Fat. Acids* **2005**, 72, 219–226.
41. Bae, Y.S.; Oh, H.; Rhee, S.G.; Yoo, Y.D. Regulation of reactive oxygen species generation in cell signaling. *Mol. Cells* **2011**, 32, 491–509.
42. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, 65, 55–63.
43. Rahbari, R.; Sheahan, T.; Modes, V.; Collier, P.; Macfarlane, C.; Badge, R. M. A novel L1 retrotransposon marker for HeLa cell line identification. *Biotechniques* **2009**, 46, 277–284.
44. Helfand, S.L.; Werkmeister, J.; Roder, J.C. Chemiluminescence response of human natural killer cells: the relationship between target cell binding, chemiluminescence, and cytolysis. *J. Exp. Med.* **1982**, 156, 492–505.
45. Choudhary, M.I.; Dur-e-Shahwar; Parveen, Z.; Abdul, J.; Ali, I. Antifungal steroidal lactones from *Withania coagulance*. *Phytochemistry* **1995**, 40, 1243–1246.