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Article

Diversity of Pharmacological Properties in Chinese and European Medicinal Plants: Cytotoxicity, Antiviral and Antitrypanosomal Screening of 82 Herbal Drugs

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Abstract: In an extensive screening, the antiviral, antitrypanosomal and anticancer properties of extracts from 82 plants used in traditional Chinese medicine and European phytomedicine were determined. Several promising plants that were highly effective against hepatitis B virus (HBV), bovine viral diarrhoea virus (BVDV)—a flavivirus used here as a surrogate *in vitro* model of hepatitis C virus, trypanosomes (*Trypanosoma brucei brucei*) and several cancer cell lines were identified. Six aqueous extracts from *Celosia cristata, Ophioglossum vulgatum, Houttuynia cordata, Selaginella tamariscina, Alpinia galanga* and *Alpinia oxyphylla* showed significant antiviral effects against BVDV without toxic effects on host embryonic bovine trachea (EBTr) cells, while *Evodia lepta*, *Hedyotis*

diffusa and *Glycyrrhiza* spp. demonstrated promising activities against the HBV without toxic effects on host human hepatoblastoma cells transfected with HBV-DNA (HepG2 2.2.15) cells. Seven organic extracts from *Alpinia oxyphylla, Coptis chinensis, Kadsura longipedunculata, Arctium lappa, Panax ginseng, Panax notoginseng* and *Saposhnikovia divaricata* inhibited *T. b. brucei.* Moreover, among fifteen water extracts that combined high antiproliferative activity (IC_{50} 0.5–20 μg/mL) and low acute *in vitro* toxicity (0–10% reduction in cell viability at IC_{50}), *Coptis chinensis* presented the best beneficial characteristics. In conclusion, traditional herbal medicine from Europe and China still has a potential for new therapeutic targets and therapeutic applications.

Keywords: anticancer drugs; gastrointestinal tumors; traditional Chinese medicine; cytotoxicity; hepatitis B; hepatitis C; HepG2 2.2.15; BVDV; *Trypanosoma brucei brucei*

1. Introduction

Traditional Chinese medicine (TCM) has a long history starting with the Shang Dynasty around 1500 BC and officially uses approximately 4773 herbs, while the number of locally used plants is probably much higher [1]. Clinical efficacy was shown in various examples, one of the best known is that of artemisinin from *Artemisia annua*, commonly used against malaria, but also effective against *T. b. brucei*, viral infections and cancer [2-8].

European medicine also has a long tradition of at least 2500 years with the two important early scholars Hippocrates and Dioscorides who described more than 400 medicinal plants 2000 years ago, many of which are still in use today [9]. Many pure therapeutic agents used in modern medicine were originally based on herbal medicine; in fact, the process of developing new drugs from European herbal medicine is still alive and important discoveries are regularly made [10,11]. Even though the theoretical concept of traditional medicine differs between Europe and China, often the same plants were and are still used in both cultures to treat the same or similar health disorders. Modern European phytotherapy also includes important herbal medicines from Africa and America.

Even though the diversity of plants and possible natural products is vast, the number of targets is usually limited (Table 1). Most natural products target proteins, biomembranes or DNA unselectively. Selective interaction is often the case when especially alkaloids mimic signal molecules and interact with receptors or enzymes. It is often possible to conclude from the type of the natural products to their most likely mode of action. Saponins and monoterpenes are active on the biomembrane, while polyphenols usually interact with proteins. Alkaloids also interact with proteins or the DNA.

The formations of covalent and of non-covalent bonds are the two modes of action that form the basis of all interactions between proteins and natural products.

The two main targets for the formation of covalent bonds are free amino and free SH groups. Aldehydes, isothiocyanates and epoxids can form covalent bonds with free amino groups while sesquiterpene lactones, disulfides (e.g., allicin), polyacetylenes and epoxides can form covalent bonds with free SH groups.

Table 1. Targets in animal cells, bacteria cells and viruses [12].

Target	Activity	Secondary metabolites
Biomembrane		
	Membrane disruption	Saponins
	Disturbance of membrane fluidity	Saponins, monoterpenes
	Disturbance of membrane proteins	Monoterpenes
Proteins (unspecific interaction)		
	Non-covalent bonding (change of 3D protein conformation)	Phenolic molecules (flavonoids, catechins, tannins, anthraquinones, quinones, lignans, phenylpropanoids)
	Covalent bonding (change of 3D protein conformation)	Allicin, furanocoumarins, isothiocyanates, sesquiterpene lactones, aldehydes, epoxids, triple bonds
Proteins (specific interaction)		
	Inhibition of enzymes	Structural mimetics of signal molecules (many alkaloids, e.g., nicotine), hydrogen cyanide from cyanogens
	Inhibition of Na^+ K^+ pumps	Cardiac glycosides
	Inhibition of microtubule formation	Colchicine, podophyllotoxin, taxol, vinblastine
	Inhibition of protein biosynthesis	Emetine, lectins
	Inhibition of transporters	Non-protein amino acids
	Modulation of hormone receptors	Isoflavonoids
	Modulation of ion channels	Many alkaloids, aconitine
	Modulation of neuroreceptors	Many alkaloids, some non-protein amino acids
	Modulation of regulatory proteins	Caffeine, phorbol esters
	Modulation of transcription factors	Structural mimetics of hormones (e.g., isoflavones genistein, daidzein)
DNA/RNA		
	Covalent modification (alkylation)	Aristolochic acids, furanocoumarins, pyrrolizidine alkaloids, molecules with epoxy groups
	Inhibition of DNA topoisomerase I	Berberine, camptothecin
	Inhibition of transcription	Amanitine
	Intercalation	Planar, aromatic and lipophilic molecules (anthraquinones, berberine, emetine, quinine, sanguinarine, furanocoumarins)

The second mechanism of maybe even greater importance due to its universality is the formation of non-covalent bonds between phenolic OH-groups and amino groups. The proton of the phenolic OH-group can partly dissociate under physiological conditions so that unspecific interactions by forming strong, ionic bonds occur with proteins. Tannins are especially effective due to their large number of hydroxyl groups.

All of these interactions will change the three dimensional structure of the protein and thus inactivate it. The omnipresence of these unspecific natural products in plants explains the efficacy of many plant extracts. They are responsible for the great number of "hits" usually occurring in extended screenings of medicinal plant extracts (Table 2).

Table 2. Main Compounds of Plants used in this study [9,13].

Family	Species	Main Compounds
Acanthaceae	*Andrographis paniculata*	Diterpenelactones
Amaranthaceae	*Celosia cristata*	Lectins (amarathin, isoamaranthin, celosianin), ferulic acid
Apiaceae	*Bupleurum chinense*	Flavonoids (quercetin, rutin, isoquercetin, isorhamnetin), β-sitosterol, β-sitosterol-3-*O*-glucosid, α-spinasterol, α-spinasterol-3-*O*-glucoside
	Bupleurum marginatum	Flavonoids (quercetin, rutin, isoquercetin, isorhamnetin), β-sitosterol, β-sitosterol-3-*O*-glucosid, α-spinasterol, α-spinasterol-3-*O*-glucoside
	Centella asiatica	Triterpenes (asiaticoside, asiatic acid, madecassic acid), flavonoids (kaempferol), monoterpenes (camphor), fatty acids (palmitic acid)
	Cnidium monnieri	Monoterpenes (pinene), cnidium lactone
	Saposhnikovia divaricata	Polyacetylenes, furanocoumarins, chromones
Araliaceae	*Eleutherococcus senticosus*	Saponins (ginsenosides), polyacetylenes, fatty acids, amino acids, polysaccharides
	Panax ginseng China	Saponins (ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1), polyacetylenes (panaxynol, panaxydol, panaxytriol, falcarindiol), fatty acids, amino acids, polysaccharides
	Panax ginseng Korea	Saponins (ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1), polyacetylenes (panaxynol, panaxydol, panaxytriol, falcarindiol), fatty acids, amino acids, polysaccharides
	Panax notoginseng	Saponins (ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1), polyacetylenes (panaxynol, panaxydol, panaxytriol, falcarindiol), fatty acids, amino acids, polysaccharides
Arecaceae	*Areca catechu*	Alkaloids (arecoline, arecaidin, arecolidin, guvacolin, guvacin)
Asclepiadaceae	*Cynanchum paniculatum*	Glucosides (cynanchocerin, cynanchin)

Table 2. *Cont.*

Family	Species	Main Compounds
Asteraceae	*Artemisia annua*	Sesquiterpene lactones (artemisinin, arteannuin, artemisitene), monoterpenes (1,8 cineol, borneol, camphor, menthol), coumarins (coumarin, scopoletin)
	Artemisia capillaris	Sesquiterpene lactones, monoterpenes (1,8 cineol, borneol, camphor, menthol), coumarins (coumarin, scopoletin)
	Arctium lappa	Monoterpenes, polyacetylenes (falcarinol), fatty acids, sterols
	Centipeda minima	Monoterpenes (thymol), terpene glycosids, sesquiterpene lactones
	Chrysanthemum indicum	Monoterpenes (1,8-cineole, pinene, borneol, camphor), tannins
	Chrysanthemum morifolium	Monoterpenes (1,8-cineole, pinene, borneol, camphor), tannins
	Eclipta prostrata	Monoterpenes, volatile compounds (Heptadecane, 6,10,14-trimethyl-2-pentadecanone, n-hexadecanoic acid, pentadecane, eudesma-4(14),11-diene, phytol, octadec-9-enoic acid, 1,2-benzenedicarboxylic acid diisooctyl ester, (Z,Z)-9,12-octadecadienoic acid)
	Senecio scandens	Pyrrolizidine alkaloids, terpenoids
	Siegesbeckia orientalis	Phytosterols (β-sitosterol)
	Taraxacum officinale	Sesquiterpene lactones, phenolic acids, triterpene saponins, inulin, phytosterols (β-sitosterol)
Berberidaceae	*Berberis bealei*	Alkaloids (berberine, columbamine, jatrorrhizine, palmatine)
	Dysosma versipellis	Flavonoids, podophyllotoxin lignans
	Epimedium koreanum	Flavonoids (quercetin, maohuoside B, epimedin A, epimedin B, icariin, icriside II, icariside I, epimedoside A, hexandraside E)
Brassicaceae	*Capsella bursa-pastoris*	Flavonoids, terpenes, glucosinolates, saponins, tannins
	Isatis indigotica (root)	Flavonoids, glucosinolates, alkaloids (isatisine A, indican, isatin, indirubin and indigotin)
	Isatis indigotica (leaf)	Flavonoids, glucosinolates, alkaloids (isatisine A, indican, isatin, indirubin, indigotin)
Caprifoliaceae	*Lonicera confusa*	Flavonoids (rutin, quercetin, luteilin-7-*O*-beta-D-galactoside, lonicerin), chlorogenic acid, beta-sitosterol, tetratriacontane)
Convallariaceae	*Polygonatum kingianum*	Flavonoids, steroidal saponins
Crassulaceae	*Rhodiola rosea*	Glucosides (salidroside, tyrosol)
Cupressaceae	*Platycladus orientalis*	Monoterpenes
Dryopteridaceae	*Cyrtomium fortunei*	Flavonoids
Ephedraceae	*Ephedra sinica*	Phenylethylamine alkaloids (ephedrine)
Equisetaceae	*Equisetum hiemale*	Flavonoids, silicic acids

Table 2. *Cont.*

Family	Species	Main Compounds
Euphorbiaceae	*Croton tiglium*	Glyceryl crotonate, crotonic acid, crotonic resin, phorbol esters (phorbol formate, phorbol butyrate, phorbol crotonate)
Fabaceae	*Abrus cantoniensis*	Lectins, indolalkaloids
	Acacia catechu	Flavonoids (quercetin, rutin), catechin, epicatechin
	Cassia tora	Flavonoids, dianthrone glycosides (sennoside A, B), anthraquinones (anthrones, emodin, rhein)
	Desmodium styracifolium	Monoterpenes, alkaloids
	Glycyrrhiza inflata	Flavonoids, isoflavonoids, chalcone (liquiritin, isoliquiritin), saponins (glycyrrhizic acid, 4-hydroxy-glycyrrhtinic acid), monoterpenes (1-(2-Furyl)propan-2-one), pyrazine (2-acetyl-1-furfuryl pyrrole), benzene (1-methoxy-4-isopropylbenzene)
	Spatholobus suberectus	Flavonoids, catechin, pyranoside
	Sutherlandia frutescens	Flavonoids, triterpene saponins, L-canavanin, pinitol
Geraniaceae	*Geranium wilfordii*	Flavonoids, tannins, monoterpenes
	Pelargonium sidoides	Flavonoids, tannins, coumarines, monoterpenes
Ginkgoaceae	*Ginkgo biloba*	Flavonoids (glycosides of kaempferol, quercetin, isorhamnetin), bisflavonoids, proanthocyanidins, ginkgolic acid, the sesqiterpene alcohol bilobalide, terpene lactones, diterpene lactones (ginkgolides)
Hypericaceae	*Hypericum japonicum*	Hypericin, hyperforin, monoterpenes, flavonoids, tannins, saponins
Iridaceae	*Belamcanda chinensis*	Flavonoids (belamcandin, iridin)
Lamiaceae	*Mentha haplocalyx*	Monoterpenes (menthol)
	Prunella vulgaris	Triterpene saponins, flavonoids (rutin) tannins, rosmarinic acid, monoterpenes (camphor)
	Scutellaria baicalensis	Flavonoids, iridoid glycosides
Lauraceae	*Cinnamomum cassia*	Monoterpenes (1,8-cineol, pinene, cinnamaldehyde), coumarins, tannins
Loranthaceae	*Taxillus chinensis*	Flavonoids (avicularin, quercetin)
Lythraceae	*Punica granatum*	Tannins (punicalin, punicalagin), piperidine alkaloids
Magnoliaceae	*Magnolia officinalis*	Tannins, flavonoids (rutin), sesquiterpenes, monoterpenes (1,8-cineol)
Melanthiaceae	*Paris polyphylla*	Steroidal saponins (dioscin, polyphyllin D)
Myrsinaceae	*Lysimachia christinae*	Flavonoids, tannins, triterpene saponins
Myrtaceae	*Eucalyptus robusta*	Monoterpenes (1,8-cineol) sesquiterpenes
Ophioglossaceae	*Ophioglossum vulgatum*	Quercetin 3-*O*-methyl ether, ophioglonin
Orchidaceae	*Dendrobium loddigesii*	Alkaloids (dendrobine, nobiline)
Paeoniaceae	*Paeonia lactiflora*	Flavonoids, (kaempferol), β-sitosterol, resveratrol derivatives, phytoestrogens, monoterpene glycosid (paeoniflorin)
Pedaliaceae	*Harpagophytum procumbens*	Iridoid glycosides (harpagide, harpagoside)

Table 2. *Cont.*

Family	Species	Main Compounds
Poaceae	*Cymbopogon distans*	Monoterpenes (1,8-cineol, pinene, cymbopogone, cymbopogonol)
Polygonaceae	*Fallopia japonica (syn. Polygonum cuspidatum)*	Anthraquinones (emodin, rhein, chrysophanol), tetrahydroxystilbene glucosides, steroidal saponins, tannins
	Fallopia multiflora (syn. Polygonum multiflorum)	Flavonoids, tannins
	Polygonum aviculare	Flavonoids, tannins
	Rheum officinale	Flavonoids, tannins, anthraquinone glycosides (emodin, rhein)
Ranunculaceae	*Coptis chinensis*	Alkaloids (berberine, palmatine, coptisine, columbamine, epiberberine)
Rosaceae	*Rosa chinensis*	Flavonoids, tannins, carotinoids, vitamin C
	Rosa laevigata	Flavonoids, tannins, carotinoids, vitamin C
	Sanguisorba officinalis	Tannins, flavonoids, saponins, proanthocyanidins
Rubiaceae	*Hedyotis diffusa*	Iridoid glycosides
Rutaceae	*Evodia lepta*	Indole alkaloids, (evodiamin, rutecarpin), chromenes
	Evodia rutaecarpa	Indole alkaloids, (evodiamin, rutecarpin)
	Phellodendron chinense	Isoquinoline alkaloids (berberine, palmatine, jatrorrhizine), sesquiterpene lactones
Saururaceae	*Houttuynia cordata*	Flavonoids (quercetin, quercetin 3-rhamnoside), norcepharadione B
Schisandraceae	*Kadsura longipedunculata*	Lignans (kadsurilignans), triterpenoid acids, triterpene dilactones, camphene, borneol
Selaginellaceae	*Selaginella tamariscina*	Flavonoids (amentoflavone, isocryptomerin, biflavonoids), sterols
Valerianaceae	*Patrinia scabiosaefolia*	Triterpene saponins, iridoid glycosides (patrinoside)
Verbenaceae	*Verbena officinalis*	Iridoid glycosides, flavonoids
Violaceae	*Viola yezoensis*	Flavonoids, saponins
Zingiberaceae	*Alpinia galanga*	Monoterpenes (camphor, cineole, d-pinene, eugenol, cadinene), flavonoids (galangin, riboflavin), niacin, 1'-acetoxychavicol acetate, ascorbic acid
	Alpinia oxyphylla	Monoterpenes (camphor, cineole, d-pinene, eugenol, cadinene), flavonoids (galangin, riboflavin), niacin, 1'-acetoxychavicol acetate, ascorbic acid

Hepatitis B and hepatitis C are responsible for 75% of all cases of liver diseases worldwide, often causing cirrhosis and hepatocellular carcinoma [14,15]. Hepatitis B and hepatitis C account for the most problematic viral infections, since the standard treatment with pegylated IFN-γ and the purine nucleoside analogues lamivudine and ribavirin have severe side effects while being at the same time ineffective for 50% of the patients [14,16]. Thus, new drugs are urgently needed [17]. Together with the bovine viral diarrhoea virus (BVDV), and the Japanese Encephalitis virus, hepatitis C virus (HCV) belongs to the Flaviviridae family. As BVDV, whose cytopathic strains induce a lytic infection in

some cell lines, such as embryonic bovine trachea (EBTr) cells, is easier to manipulate and lacks human infectivity, this is commonly used as *in vitro* model for infections of this viral family [18].

Our knowledge of the natural products of many plants used in European and Chinese phytomedicine is broad (Table 2), however, many new discoveries are still possible. Previously, several studies demonstrated the promising potential of traditional phytomedicine for the discovery of new antiviral drugs. Artemisinin and related compounds proved effective in screening assays against viral hepatitis [6,7,19]. In water extracts of *Terminalia chebula*, *Sanguisorba officinalis*, *Rubus coreanus* and *Rheum palmatum,* Kim *et al.* [20] discovered prominent anti-hepatitis B virus (HBV) activities. The ethanolic extract of *Hypericum perforatum,* a well-established drug for treatment of depression [9] was also shown to be active against the HBV [21]. Laxative anthraquinones isolated from *Rheum palmatum* demonstrated significant effects against HBV [22] and saikosaponins from *Bupleurum* species were previously shown to lower significantly the HBV level in the HepG2 2.2.15 assay [23]. HepG2 2.2.15 is a stable cell line infected with the HBV. The assay measures the production of secreted HBV from the cell by using real time quantitative PCR.

Parasites such as protozoa and helminths cause a major health threat in many tropical countries [24], while suitable drugs are still rare [25]. Blood parasites of the genus *Trypanosoma* (*Trypanosoma brucei rhodesiense* and *T. b. gambiense*) are responsible for African trypanosomiasis (sleeping sickness) with serious consequences for human health and economy. Due to the high infectivity of African human trypanosomes, *T. b. brucei* is commonly used as model organism with similar morphology and biochemical processes, while being only infective for cattle [24,26,27]. This subspecies causes the cattle epidemic nagana, it is responsible for severe financial loss of 1340 billion USD per year [28].

Currently, only four drugs are approved internationally for the treatment of humans against sleeping sickness: suramin, pentamidine, melarsoprol and eflornithine. Diminazene, another effective antitrypanosomal drug, is only approved for the use on animals because of severe side effects [24]. Even the drugs approved for human use are responsible for serious side effects, and furthermore, the parasites develop increasing resistance to them [29-32]. This situation makes the discovery of new, effective drugs an urgent task of the 21st century [33-35].

When considered together, enterohepatic tumors, *i.e.*, those affecting the liver, the biliary duct, gallbladder and the intestine, constitute the first cause of death due to cancer. Although in many cases surgery and radiotherapy are efficacious, these therapeutic strategies cannot always be applied. Moreover, even when the removal of tumors is possible, pre- and post-operative pharmacological adjuvant regimens are often needed. However, one important limitation to the use of cytostatic drugs to treat enterohepatic tumors is that they generally exhibit marked resistance to currently available pharmacological approaches and the development of resistance during treatment [36].

Many natural products and derivatives thereof belong to the standard repertoire of cancer chemotherapy. Examples are Vinca alkaloids, such as vincristine, vinblastine and vinorelbine, obtained from Madagascar periwinkle (*Catharanthus rosea*). Also taxanes such as paclitaxel and docetaxel, which are produced from the bark of Pacific yew (*Taxus*), podophyllotoxins, such as etoposide and teniposide, derivatives of the genus *Podophyllum*, and camptothecin, derived from the Asian "Tree of Happiness" (*Camptotheca acuminata*) and its derivatives, irinotecan and topotecan, are natural products from TCM plants [4].

In this study, extracts from 82 traditional medicinal plants were screened against HBV and flaviviruses, *T. b. brucei* and several cancer cell lines. Our aim was to detect new sources of active compounds for the possible treatment of these important causes of diseases.

2. Experimental Section

2.1. Chemicals

Dimethylsulfoxide (DMSO), trypsin-EDTA, DMEM and MEM with GLUTAMAX media, fetal bovine serum (FBS) and supplementary chemicals were bought from Gibco® Invitrogen; Germany. Antibiotic/antimycotic solution, gentamicin, Neutral Red (NR, 3-amino-7-dimethylamino-2-methylfenazine), $NaHCO_3$, L-glutamine and MEM media were purchased from Sigma-Aldrich (Madrid, Spain). Geneticin® (G418) was from Roche (Barcelona, Spain). Dried TCM plants were obtained in Shanghai; South African plants were provided by Prof. van Wyk, University of Johannesburg, South Africa.

2.2. Authentication of Plant Material

The TCM plants were genetically identified by DNA barcoding to confirm the identity and to exclude adulterations. DNA was isolated from plant drugs; their chloroplast *rbc*L gene was amplified and sequenced. The obtained sequences were authenticated with sequences obtained from sample species of the Botanical Garden of Heidelberg and databases. Voucher specimens of the plant material were deposited at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany. Additionally, the plants were authenticated by visual and microscopic techniques.

2.3. Extract Preparation

Five hundred grams of dry plant material was powdered and extracted with dichloromethane, methanol and water under moderate heat using a reflux condenser for 4 hours. The extracts obtained were concentrated using the rotation evaporator, stored at −40 °C under exclusion of light and dried under vacuum prior to the experiments. Dried extracts were dissolved in DMSO for the experiments.

2.4. Test Organisms

T. b. brucei TC 221 were originally obtained from Prof. Peter Overath (Max-Plank Institut für Biologie, Tübingen) by Dr. D. Steverding before being cultured at the IPMB, Heidelberg since 1999. HeLa cancer cells and Cos7 fibroblast cells (African green monkey kidney cells immortalized with the monkey virus SV40) were cultured at the IPMB, Heidelberg for several years; Hep G2, SK-Hep1 and LS 174T, HepG2 2.2.15 and EBTr cells were cultured at the Laboratory of Experimental Hepatology and Drug Targeting (HEVEFARM), University of Salamanca, CIBERehd, Spain.

2.5. Methods

Cancer Cells (HeLa, Hep G2, SK-Hep1 and LS 174 T) were basically grown as previously described [37], HeLa and Cos7 cells were grown at 37 °C with 5% CO_2 in DMEM complete media (10% heat-inactivated FBS; 5% penicillin/streptomycin; 5% non-essential amino acids). Hep G2, SK-Hep1, and LS 174T cells were grown at 37 °C with 5% CO_2 in MEM complete media (10% heat-inactivated FBS; 1% antibiotic-antimycotic solution).

HepG 2.2.15 cells were cultured as previously described [7] in DMEM complete medium with 10% FBS, geneticin and gentamicin. EBTr cells were cultured as described elsewhere [6], they were maintained in MEM-GLUTAMAX medium with 10% heat-inactivated FBS; 1% penicillin/streptomycin, and 0.1% gentamicin.

T. b. brucei TC221 cells were cultured in BALTZ medium [38] supplemented with 20% inactivated FBS and 0.001% β-mercaptoethanol.

The MTT cell viability assay was used to determine cytotoxicity in Cos7 and HeLa cells [39,40]. Cells during the logarithmic growth period were seeded in 96 well plates (Greiner Labortechnik) at concentrations of 2×10^4 cells/well and grown for 24 h. Dried and powdered extracts were dissolved in DMSO before being serially diluted to 10 concentrations in 96 well plates. Cells were incubated with the extract for 24 h before the medium was removed and replaces with fresh medium containing 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The formazan crystals were dissolved in DMSO 4 h later; the absorbance was measured at 570 nm with a Tecan Safire II Reader.

T. b. brucei TC221 cell viability was additionally to the MTT assay confirmed and evaluated using microscopic techniques.

Toxicity of the extracts for *T. b. brucei* was compared to HeLa and Cos7 cells and the Selectivity index (SI) was calculated. SI: ratio of the IC_{50} value of mammalian cells divided by the IC_{50} value of trypanosomes.

To test the antiproliferative effect, 5×10^3 or 15×10^3 cells per well (depending on the cell line) were seeded in 96 well plates and incubated with 5, 10, 25, 50, 100, and 200 µg/mL water extract for 72 h. The cell viability was also determined using the MTT assay with minor modifications. Acute toxicity was similarly measured using MTT assay but after short-term (6 h) incubation with the extracts at the concentrations of IC_{50} calculated for each cell line.

To determine the antiviral effect of the extracts, BVDV was used here as a substitute *in vitro* model for HCV infection. Bovine epithelial cells obtained from embryonic trachea (EBTr) were cultured in MEM with GLUTAMAX medium as described previously [6]. They were seeded in 96 well plates (15×10^3 cells/well; 50 µL/well) and left to attach for 2 h. Afterwards, the cells were infected with 50 µL/well of the desired dilution in culture medium of an initial suspension of BVDV (cytopathic strain Oregon C24V, genotype I, subgenotype b) to reach 40% cytopathic effect. After 48 h of incubation the medium was replaced with dilutions in culture medium of the extracts (1, 5, 10, 50, 100 µg/mL). The viability of the EBTr cells was measured using the MTT assay after 72 h incubation.

An HBV antiviral assay based on the HepG2 2.2.15 model was used to determine the antiviral activity of the extracts [41]. HepG2 2.2.15 cells were seeded in six-wells plates (35×10^4 per well) before being incubated for 21 days with 50 µg/mL, 25 µg/mL and 12.5 µg/mL extract. The culture

medium was replaced every 3 days with fresh medium, containing the extract dilutions. Quantitative real-time PCR (QPCR) was used to measure the HBV-DNA levels in the culture medium (representing HBV virion production) as described previously [7]. Cytotoxicity was determined using the uptake of NR dye at the end of treatment [42].

At least three cultures for each experimental condition were carried out. Data points were obtained in triplicate form (*T. b. brucei*, cancer cell lines, Cos7, HepG2 2.2.15 cells) and in 8 different wells (EBTr). The IC_{50} value was calculated using SigmaPlot® 11.0 (4 parameter logistic curve). Statistical significance determined via paired t-test or the Bonferroni method of multiple-range testing.

3. Results and Discussion

The great diversity of natural products occurring plants is of the utmost importance for the discovery of new pharmaceutical lead compounds. Through millions of years of evolution the defence mechanisms of plants were perfected. The great variety of natural products clearly demonstrates the efficacy of this defence strategy against herbivores, but also fungi, bacteria and viruses (Table 2). In many cases the plants do not rely on specific interactions but also rely on unspecific molecules that interact with a great number of targets (Table 1). Of highest importance are the interactions with free amino and free SH groups. While aldehydes, isothiocyanates and epoxids are able to form covalent bonds with free amino groups, sesquiterpene lactones, disulfides, polyacetylenes and epoxides interact with free SH groups. Phenolic OH-groups interact on a non-covalent basis with free amino groups by forming strong hydrogen and ionic bonds.

The cytotoxicity of water and organic solvent extracts from 82 medicinal plants was determined in the fibroblast cells Cos7 and in four cancer cell lines: HeLa, HepG2, SK-Hep1 and LS 174T (Tables 3 and 4). The aqueous extracts were also screened against BVDV and HBV (Table 3), whereas organic solvent extracts were assayed on *T. b. brucei* (Table 4). Our results revealed promising results in order to use several of these plants as sources for therapeutic agents.

The viral particles offer three main targets to the natural products (Table 1). They can interact with the surface proteins, the biomembrane and the DNA or RNA. While most plants interact unselectively with the virus, selective interactions do also occur.

10 plants demonstrated antiviral protection against BVDV in combination with low cytotoxicity. Four plants (*Panax ginseng, Cassia tora, Ginkgo biloba* and *Viola yezoensis*) exerted protective antiviral effect only at high doses, whereas other six plant extracts (*Celosia cristata, Ophioglossum vulgatum, Houttuynia cordata, Selaginella tamariscina, Alpinia galanga* and *Alpinia oxyphylla*) were effective at lower concentrations (Table 3).

Regarding the six plants with higher potential interest as a source of anti-HCV drugs, antiviral glycoproteins, CCP-25 and CCP-27, purified from the leaves of *Celosia cristata* [43] have been previously studied [44-48]. Their ability to inhibit viral RNA translation activities against several plant viruses have been described [49].

Table 3. Cytotoxicity against cancer cells, Cos7 fibroblasts, and antiviral activity against HBV and flaviviruses of water extracts obtained from 82 medicinal plants.

Family	Species	IPMB/No.	GenBank	Antiumor Effect					Antiviral Effect			
				Cos 7 IC_{50} (µg/mL)	HeLa IC_{50} (µg/mL)	HepG2 IC_{50} (µg/mL)	SK-Hep1 IC_{50} (µg/mL)	LS 174T IC_{50} (µg/mL)	[a]anti-BVDV Toxicity on EBTr cells	[b]anti-BVDV protection in EBTr cells	[c]anti-HBV effect in Hep G2 2.2.15	[d]Toxicity at effective doses
Acanthaceae	*Andrographis paniculata*	P6838/04	JF949965	255.6	576.0	170	80	>200	0	0	++	++
Amaranthaceae	*Celosia cristata*	P6848/14	JF949970	263.9	2773.5	200	180	>200	0	++	0	ND
Apiaceae	*Bupleurum chinense*	P6844/10	JF950021	15.6	339.3	120	100	100	++	0	ND	ND
	Bupleurum marginatum	P6845/11	JF949968	350.6	838.1	ND	ND	ND	ND	ND	ND	ND
	Centella asiatica	P6849/15	JF950022	325.8	1436.8	>200	40	70	++++	0	0	ND
	Cnidium monnieri	P6854/20	JF949973	339.7	775.5	>200	>200	>200	++++	0	0	ND
	Saposhnikovia divaricata	P6902/68	JF949988	153.0	1024.7	>200	200	155	++	0	++	++
Araliaceae	*Eleutherococcus senticosus*	P6919/79	-	130.5	430.0	>200	125	160	++	++	0	ND
	Panax ginseng	P8088/81	JF950028	151.7	2594.6	>200	140	>200	0	+	0	0
	Panax notoginseng	P6887/53	JF950030	182.3	1574.9	>200	>200	200	0	0	ND	ND
Arecaceae	*Areca catechu*	P6840/06	-	16.6	378.1	40	21	90	++++	0	ND	ND
Asclepiadaceae	*Cynanchum paniculatum*	P6858/24	JF949975	220.7	693.9	>200	130	>200	++	++	0	ND

Table 3. *Cont.*

Family	Species	IPMB/No.	GenBank	Antiumor Effect					Antiviral Effect			
				Cos 7 IC_{50} (µg/mL)	HeLa IC_{50} (µg/mL)	HepG2 IC_{50} (µg/mL)	SK-Hep1 IC_{50} (µg/mL)	LS 174T IC_{50} (µg/mL)	[a]anti-BVDV Toxicity on EBTr cells	[b]anti-BVDV protection in EBTr cells	[c]anti-HBV effect in Hep G2 2.2.15	[d]Toxicity at effective doses
Asteraceae	*Artemisia annua*	P6841/07	JF949966	288.6	775.5	177	50	>200	++++	0	++	++
	Artemisia capillaris	P6842/08	JF949967	201.9	561.7	142	30	>200	++	0	++	++
	Arctium lappa	P6839/05	JF949994	355.5	516.3	200	5	>200	++	0	0	ND
	Centipeda minima	P6850/16	-	55.6	207.2	72	0,5	130	++	++	ND	ND
	Chrysanthemum indicum	P6851/17	JF949971	320.9	583.4	130	8	200	++++	0	0	ND
	Chrysanthemum morifolium	P6852/18	JF949972	760.4	1045.8	>200	180	>200	++	0	0	ND
	Eclipta prostata	P6863/29	JF950000	291.7	667.0	>200	30	120	++	++	0	ND
	Senecio scandens	P6905/71	JF949989	114.2	607.9	3.5	50	25	++++	++	ND	ND
	Siegesbeckia orientalis	P6906/72	JF949990	159.2	542.8	40	100	50	++	++	ND	ND
	Taraxacum officinale	P6908/74	JF950019	156.9	708.5	130	147	65	++	++	0	ND
Berberidaceae	*Berberis bealei*	P6883/49	JF949996	270.0	659.4	63	60	70	ND	ND	ND	ND
	Dysosma versipellis	P6862/28	-	1276.9	1274.8	>200	3.5	3.5	++++	0	0	ND
	Epimedium koreanum	P6865/31	JF950002	140.7	280.2	5	32	10	++	0	ND	ND
Brassicaceae	*Isatis indigotica* (root)	P6877/43	JF949981	557.2	2427.4	>200	> 200	>200	0	0	0	ND
	Isatis indigotica (leaf)	P6878/44	JF949981	93.5	1223.5	170	98	80	++	++	0	ND
Caprifoliaceae	*Lonicera confusa*	P6880/46	JF949982	446.8	812.2	>200	>200	>200	++	0	0	ND
Convallariaceae	*Polygonatum kingianum*	P6892/58	JF950027	298.4	2321.7	>200	130	42	++	0	0	ND
Crassulaceae	*Rhodiola rosea*	P6920/84	-	61.9	144.4	160	110	40	++	++	0	ND
Cupressaceae	*Platycladus orientalis*	P6891/57	JF950011	97.7	428.2	>200	155	10	++	0	0	ND

Table 3. *Cont.*

Family	Species	IPMB/No.	GenBank	Antiumor Effect					Antiviral Effect			
				Cos 7 IC_{50} (μg/mL)	HeLa IC_{50} (μg/mL)	HepG2 IC_{50} (μg/mL)	SK-Hep1 IC_{50} (μg/mL)	LS 174T IC_{50} (μg/mL)	[a]anti-BVDV Toxicity on EBTr cells	[b]anti-BVDV protection in EBTr cells	[c]anti-HBV effect in Hep G2 2.2.15	[d]Toxicity at effective doses
Dryopteridaceae	*Cyrtomium fortunei*	P6859/25	JF949998	30.4	567.4	>200	>200	>200	++	++	0	ND
Ephedraceae	*Ephedra sinica*	P6864/30	JF950001	69.1	193.1	200	150	>200	++++	++	ND	ND
Equisetaceae	*Equisetum hiemale*	P6866/32	JF950003	265.9	1058.2	5	>200	>200	++	0	ND	ND
Euphorbiaceae	*Croton tiglium*	P6856/22	-	166.2	1052.7	140	50	>200	++++	0	++	++
Fabaceae	*Abrus cantoniensis*	P6835/01	JF949964	575.2	587.1	>200	100	>200	++	++	0	ND
	Acacia catechu	P6836/02	-	35.7	157.5	>200	25	>200	++	++	++	++
	Cassia tora	P6847/13	JF949969	481.3	1519.3	>200	0.5	>200	0	+	++	++
	Desmodium styracifolium	P6861/27	JF949976	333.5	651.4	>200	>200	150	0	0	0	ND
	Glycyrrhiza inflata	P6873/39	JF950025	583.9	2288.0	>200	>200	185	0	0	++++	0
	Spatholobus suberectus	P6907/73	JF949991	16.6	174.1	100	135	70	++	0	ND	ND
	Sutherlandia frutescens	tba/83	-	857.6	1670.7	>200	70	>200	0	0	0	ND
Geraniaceae	*Geranium wilfordii*	P6867/33	JF949977	225.8	62.1	80	45	200	++	++	ND	ND
	Pelargonium sidoides	tba/82	-	15.2	62.2	200	>200	45	++	0	0	ND
Ginkgoaceae	*Ginkgo biloba*	P6872/38	JF950005	450.8	1717.0	> 200	9	>200	0	+	0	ND
Hypericaceae	*Hypericum japonicum*	P6876/42	JF949980	151.8	445.5	165	80	100	++	++	0	ND
Iridaceae	*Belamcanda chinensis*	P6843/09	JF949995	222.1	1378.8	>200	>200	>200	++	0	++	++
Lamiaceae	*Mentha haplocalyx*	P6884/50	JF949984	285.7	519.1	70	82	>200	++++	0	ND	ND
	Prunella vulgaris	P6896/62	JF950013	21.5	341.3	100	145	80	++	0	ND	ND
	Scutellaria baicalensis	P6903/69	JF950017	46.4	150.0	80	50	120	++	0	ND	ND

Table 3. *Cont.*

Family	Species	IPMB/No.	GenBank	Antiumor Effect					Antiviral Effect			
				Cos 7 IC_{50} (μg/mL)	HeLa IC_{50} (μg/mL)	HepG2 IC_{50} (μg/mL)	SK-Hep1 IC_{50} (μg/mL)	LS 174T IC_{50} (μg/mL)	[a]anti-BVDV Toxicity on EBTr cells	[b]anti-BVDV protection in EBTr cells	[c]anti-HBV effect in Hep G2 2.2.15	[d]Toxicity at effective doses
Lauraceae	*Cinnamomum cassia*	P6853/19	JF950023	453.9	713.6	180	>200	>200	++++	0	++	++
Loranthaceae	*Taxillus chinensis*	P6909/75	JF949992	181.7	1023.2	>200	>200	155	0	0	0	ND
Lythraceae	*Punica granatum*	P6897/63	JF950014	8.6	152.4	100	100	60	++++	0	ND	ND
Magnoliaceae	*Magnolia officinalis*	P6882/48	JF950008	73.0	451.5	ND	ND	ND	ND	ND	ND	ND
Melanthiaceae	*Paris polyphylla*	P6888/54	JF950010	38.4	42.6	54	168	8	++++	++	ND	ND
Myrsinaceae	*Lysimachia christinae*	P6881/47	JF949983	152.1	431.4	>200	>200	>200	++	0	0	ND
Myrtaceae	*Eucalyptus robusta*	P6868/34	-	94.1	15.8	ND	ND	ND	ND	ND	ND	ND
Ophioglossaceae	*Ophioglossum vulgatum*	P6885/51	JF950009	344.0	1780.1	>200	>200	>200	0	++	0	ND
Orchidaceae	*Dendrobium loddigesii*	P6860/26	JF949999	104.0	294.4	>200	70	160	++	++	0	ND
Paeoniaceae	*Paeonia lactiflora*	P6886/52	JF950026	148.2	287.3	>200	>200	10	0	0	ND	ND
Pedaliaceae	*Harpagophytum procumbens*	tba/80	-	242.9	733.4	160	190	100	++	++	0	ND
Poaceae	*Cymbopogon distans*	P6857/23	JF949974	257.7	486.1	>200	>200	>200	++++	0	++	++
Polygonaceae	*Fallopia japonica*	P6894/60	JF950004	39.8	596.4	>200	>200	80	++++	++	0	ND
	Polygonum aviculare	P6893/59	JF950012	82.6	488.6	>200	>200	10	++	0	0	ND
	Polygonum multiflorum	P6895/61	JF949987	61.3	928.0	ND	ND	ND	ND	ND	ND	ND
	Rheum officinale	P6898/64	JF950015	51.5	670.9	200	25	200	++	0	0	ND
Ranunculaceae	*Coptis chinensis*	P6855/21	JF950024	118.3	101.0	10	2	18	++++	0	ND	ND

Table 3. *Cont.*

Family	Species	IPMB/No.	GenBank	Antiumor Effect					Antiviral Effect			
				Cos 7 IC_{50} (μg/mL)	HeLa IC_{50} (μg/mL)	HepG2 IC_{50} (μg/mL)	SK-Hep1 IC_{50} (μg/mL)	LS 174T IC_{50} (μg/mL)	[a]anti-BVDV Toxicity on EBTr cells	[b]anti-BVDV protection in EBTr cells	[c]anti-HBV effect in Hep G2 2.2.15	[d]Toxicity at effective doses
Rosaceae	*Rosa chinensis*	P6899/65	-	24.3	135.8	ND	ND	ND	ND	ND	ND	ND
	Rosa laevigata	P6900/66	-	93.6	781.7	190	135	60	++	++	0	ND
	Sanguisorba officinalis	P6901/67	JF950016	20.5	87.0	ND	ND	ND	ND	ND	ND	ND
Rubiaceae	*Hedyotis diffusa*	P6874/40	JF949979	158.7	1542.7	>200	>200	5	++	++	++++	0
Rutaceae	*Evodia lepta*	P6869/35	JF949978	419.2	971.0	>200	20	>200	++++	0	++++	0
	Evodia rutaecarpa	P6870/36	-	1176.9	185.6	>200	25	100	++++	++	0	ND
	Phellodendron chinense	P6890/56	JF949986	282.9	750.3	50	10	85	++	++	ND	ND
Saururaceae	*Houttuynia cordata*	P6875/41	JF950006	633.2	2835.9	>200	135	5	0	++	0	ND
Schisandraceae	*Kadsura longipedunculata*	P6879/45	JF950007	6.8	167.6	>200	20	20	++	++	0	ND
Selaginellaceae	*Selaginella tamariscina*	P6904/70	JF950018	103.9	703.4	>200	>200	200	0	++	0	0
Valerianaceae	*Patrinia scabiosaefolia*	P6889/55	JF949985	147.3	525.5	168	87	35	++	++	0	ND
Verbenaceae	*Verbena officinalis*	P6910/76	JF950020	93.9	416.9	100	168	117	++	0	ND	ND
Violaceae	*Viola yezoensis*	P6911/77	JF949993	135.0	1459.2	170	200	140	0	+	ND	ND
Zingiberaceae	*Alpinia galanga*	P6837/03	-	952.8	2357.3	>200	>200	>200	0	++	++	++
	Alpinia oxyphylla	P6917/78	-	105.8	1802.2	>200	>200	155	0	++	0	ND

[a] Toxicity on EBTr cells: 0, not toxic; ++, toxic at high concentrations; ++++, toxic in all concentrations; [b] Anti-BVDV protection in EBTr cells: 0, without effect; +, protection at high concentrations; ++, protection at low concentrations; [c] Anti-HBV effect in HepG2 2.2.15 cells: 0, without effect; ++, effect comparable to toxicity; ++++, high ability to reduce HBV-DNA; [d] Toxicity at effective dose on HepG2 2.2.15 cells: 0, not toxic; ++, effect comparable to reduction in HBV DNA. ND: Not determined.

Table 4. Cytotoxicity against HeLa cancer cells, Cos7 fibroblasts and *Trypanosoma brucei brucei* of organic extracts obtained from 82 medicinal plants.

Family	Species	IPMB/No.	GenBank	CH_2Cl_2 HeLa	CH_2Cl_2 Cos 7	CH_2Cl_2 *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*	MeOH HeLa	MeOH Cos 7	MeOH *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*
Acanthaceae	*Andrographis paniculata*	P6838/04	JF949965	188.4	104.7	16.8	11	6	323.3	344.7	28.8	11	12
Amaranthaceae	*Celosia cristata*	P6848/14	JF949970	472.0	136.0	55.2	9	2	499.8	28.4	77.2	6	0.3
Apiaceae	*Bupleurum chinense*	P6844/10	JF950021	235.2	87.1	17.0	14	5	646.4	358.7	120.8	5	3
	Bupleurum marginatum	P6845/11	JF949968	176.0	67.4	16.2	11	4	1147.0	576.0	111.9	10	5
	Centella asiatica	P6849/15	JF950022	175.0	64.9	14.0	13	4	773.0	392.8	44.7	17	8
	Cnidium monnieri	P6854/20	JF949973	127.1	37.0	14.9	9	2	251.1	120.0	17.9	14	6
	Saposhnikovia divaricata	P6902/68	JF949988	410.1	45.9	5.1	80	9	1515.6	1575.4	999.5	2	1
Araliaceae	*Eleutherococcus senticosus*	P6919/79	-	300.0	61.4	13.5	22	4	692.0	190.1	17.3	40	11
	Panax ginseng	P8088/81	JF950028	152.4	47.7	0.9	169	53	1427.9	510.8	319.0	4	1
	Panax notoginseng	P6887/53	JF950030	263.0	6.4	0.9	292	7	1241.6	229.5	469.6	2	0.4
Arecaceae	*Areca catechu*	P6840/06	-	1023.3	117.0	22.5	45	5	414.2	31.0	118.1	4	0.2
Asclepiadaceae	*Cynanchum paniculatum*	P6858/24	JF949975	395.6	114.2	53.1	7	2	500.5	227.7	39.3	13	5
Asteraceae	*Artemisia annua*	P6841/07	JF949966	107.9	34.5	8.1	13	4	287.2	201.1	51.2	6	4
	Artemisia capillaris	P6842/08	JF949967	93.5	29.4	10.6	9	3	314.9	215.4	51.9	6	4
	Arctium lappa	P6839/05	JF949994	345.0	344.2	3.6	96	96	1467.7	1813.0	2229.0	0.7	0.8
	Centipeda minima	P6850/16	-	63.3	10.4	2.2	29	5	219.1	54.2	13.3	16	4.0
	Chrysanthemum indicum	P6851/17	JF949971	152.1	63.5	16.0	10	4	355.7	287.2	15.3	23	18
	Chrysanthemum morifolium	P6852/18	JF949972	129.4	42.8	19.3	7	2	349.2	166.7	24.9	14	6
	Eclipta prostata	P6863/29	JF950000	266.4	112.0	38.1	7	3	329.7	186.1	39.6	8	4.6
	Senecio scandens	P6905/71	JF949989	268.6	143.5	13.1	21	11	299.3	126.2	18.6	16	6
	Siegesbeckia orientalis	P6906/72	JF949990	101.5	17.7	7.9	13	2	237.5	84.4	12.3	19	6
	Taraxacum officinale	P6908/74	JF950019	232.8	177.1	17.5	13	10	636.7	485.3	64.9	10	7

Table 4. *Cont.*

Family	Species	IPMB/No.	GenBank	CH_2Cl_2 HeLa	CH_2Cl_2 Cos 7	CH_2Cl_2 *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*	MeOH HeLa	MeOH Cos 7	MeOH *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*
Berberidaceae	*Berberis bealei*	P6883/49	JF949996	93.8	13.3	5.9	16	2	149.7	35.3	7.8	19	4
	Dysosma versipellis	P6862/28	-	213.9	49.9	39.5	5	1	385.2	54.9	53.2	7	1.0
	Epimedium koreanum	P6865/31	JF950002	48.7	3.5	4.2	12	0.8	257.5	30.7	12.6	20	2
Brassicaceae	*Isatis indigotica* (root)	P6877/43	JF949981	196.4	42.3	2.9	68	14	674.3	324.4	94.6	7	3
	Isatis indigotica (leaf)	P6878/44	JF949981	321.5	0.6	45.3	7	0.01	274.2	90.6	14.6	19	6
Caprifoliaceae	*Lonicera confusa*	P6880/46	JF949982	226.5	58.9	16.2	14	3	923.5	118.9	38.0	24	3
Convallariaceae	*Polygonatum kingianum*	P6892/58	JF950027	279.6	53.9	52.6	5	1	1517.9	1535.3	119.5	13	12
Crassulaceae	*Rhodiola rosea*	P6920/84	-	164.1	74.6	43.9	4	1	-	87.4	-	-	-
Cupressaceae	*Platycladus orientalis*	P6891/57	JF950011	121.7	21.8	17.7	7	1	705.5	158.2	84.2	8	1
Dryopteridaceae	*Cyrtomium fortunei*	P6859/25	JF949998	572.4	132.1	37.1	15	3	722.0	348.7	61.0	12	5
Ephedraceae	*Ephedra sinica*	P6864/30	JF950001	95.3	41.8	20.9	5	2	163.5	36.7	23.4	7	1
Equisetaceae	*Equisetum hiemale*	P6866/32	JF950003	125.6	35.7	30.9	4	1	241.2	243.5	51.6	4	4
Euphorbiaceae	*Croton tiglium*	P6856/22	-	422.9	225.9	86.5	5	2	297.0	222.1	150.4	2	1
Fabaceae	*Abrus cantoniensis*	P6835/01	JF949964	494.4	129.4	14.5	34	9	612.4	733.1	73.5	8	10
	Acacia catechu	P6836/02	-	164.1	31.5	13.1	12	2	318.0	34.8	50.8	6	0.6
	Cassia tora	P6847/13	JF949969	1335.4	189.1	185.9	7	1	670.9	75.9	276.9	2	0.2
	Desmodium styracifolium	P6861/27	JF949976	156.0	139.8	16.3	10	8	324.3	104.1	40.1	8	2
	Glycyrrhiza inflata	P6873/39	JF950025	26.4	6.9	6.4	4	1	528.3	126.8	39.0	14	3
	Spatholobus suberectus	P6907/73	JF949991	299.1	154.6	25.4	12	6	237.5	54.8	67.8	4	0.8
	Sutherlandia frutescens	tba/83	-	367.7	259.3	41.8	9	6	586.6	352.0	87.4	7	4
Geraniaceae	*Geranium wilfordii*	P6867/33	JF949977	99.1	17.0	23.0	4	0.7	236.0	169.8	13.3	18	12
	Pelargonium sidoides	tba/82	-	488.2	218.2	52.1	9	4	112.3	95.7	18.3	6	5
Ginkgoaceae	*Ginkgo biloba*	P6872/38	JF950005	768.3	15.3	71.9	11	0.2	302.9	260.1	39.3	8	6
Hypericaceae	*Hypericum japonicum*	P6876/42	JF949980	163.3	10.8	21.3	8	0.5	177.5	100.9	23.6	8	4

Table 4. *Cont.*

Family	Species	IPMB/No.	GenBank	CH_2Cl_2 HeLa	CH_2Cl_2 Cos 7	CH_2Cl_2 *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*	MeOH HeLa	MeOH Cos 7	MeOH *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*
Iridaceae	*Belamcanda chinensis*	P6843/09	JF949995	324.4	89.2	22.3	15	4	522.6	319.5	80.2	7	4
Lamiaceae	*Mentha haplocalyx*	P6884/50	JF949984	108.5	34.1	14.7	7	2	375.0	147.8	16.2	23	9
	Prunella vulgaris	P6896/62	JF950013	282.1	90.4	13.2	21	7	475.4	494.5	25.1	19	19
	Scutellaria baicalensis	P6903/69	JF950017	90.9	287.9	7.4	12	39	367.6	28.8	86.2	4	0.3
Lauraceae	*Cinnamomum cassia*	P6853/19	JF950023	138.9	23.2	11.0	13	2	272.4	108.4	13.4	20	8
Loranthaceae	*Taxillus chinensis*	P6909/75	JF949992	417.8	68.6	27.2	15	2	1213.4	378.2	59.2	20	6
Lythraceae	*Punica granatum*	P6897/63	JF950014	583.3	126.6	14.6	40	8	211.2	218.6	8.1	26	27
Magnoliaceae	*Magnolia officinalis*	P6882/48	JF950008	23.6	5.4	0.9	26	6	49.1	13.1	4.3	11	3
Melanthiaceae	*Paris polyphylla*	P6888/54	JF950010	952.6	24.0	73.6	13	0.3	35.0	5.5	11.8	3	0.4
Myrsinaceae	*Lysimachia christinae*	P6881/47	JF949983	53.4	137.3	20.6	3	7	1752.6	436.3	52.1	34	8
Myrtaceae	*Eucalyptus robusta*	P6868/34	-	-	-	-			181.4	15.2	16.3	11	1
Ophioglossaceae	*Ophioglossum vulgatum*	P6885/51	JF950009	188.9	62.8	19.8	10	3	469.0	68.8	33.2	14	2
Orchidaceae	*Dendrobium loddigesii*	P6860/26	JF949999	83.0	25.7	13.5	6	2	232.8	61.6	27.6	8	2
Paeoniaceae	*Paeonia lactiflora*	P6886/52	JF950026	166.9	34.0	9.1	18	3	294.6	309.8	11.7	25	26
Pedaliaceae	*Harpagophytum procumbens*	tba/80	-	36.2	15.8	0.9	40	17	692.6	217.2	21.4	32	10
Poaceae	*Cymbopogon distans*	P6857/23	JF949974	425.9	114.5	31.1	14	3	98.8	17.6	18.9	5	1
Polygonaceae	*Fallopia japonica*	P6894/60	JF950004	88.0	2.8	13.1	7	0.2	317.3	19.5	19.0	17	1
	Polygonum aviculare	P6893/59	JF950012	118.5	53.3	18.2	7	3	342.3	226.5	49.1	7	4
	Polygonum multiflorum	P6895/61	JF949987	469.4	107.7	98.6	5	1	437.4	48.8	62.1	7	0.7
	Rheum officinale	P6898/64	JF950015	22.5	-	34.0	0.6	-	270.9	35.3	24.5	11	1
Ranunculaceae	*Coptis chinensis*	P6855/21	JF950024	100.0	39.5	12.9	8	3	81.8	3.7	0.4	205	9

Table 4. *Cont.*

Family	Species	IPMB/No.	GenBank	CH_2Cl_2 HeLa	CH_2Cl_2 Cos 7	CH_2Cl_2 *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*	MeOH HeLa	MeOH Cos 7	MeOH *T. b. brucei*	Ratio HeLa/*T. b. brucei*	Ratio Cos7/*T. b. brucei*
Rosaceae	*Rosa chinensis*	P6899/65	-	559.4	141.5	20.1	28	7	266.6	36.7	12.5	21	3
	Rosa laevigata	P6900/66	-	712.3	151.8	20.6	35	7	1855.4	1100.1	102.9	18	10
	Sanguisorba officinalis	P6901/67	JF950016	66.5	26.7	12.3	5	2	158.5	41.6	4.0	40	10
Rubiaceae	*Hedyotis diffusa*	P6874/40	JF949979	147.8	45.3	13.3	11	3	796.1	418.1	24.9	32	16
Rutaceae	*Evodia lepta*	P6869/35	JF949978	232.0	42.0	13.9	17	3	350.7	427.7	44.4	8	9
	Evodia rutaecarpa	P6870/36	-	50.4	8.7	16.8	3	0.5	297.4	178.5	29.5	10	6
	Phellodendron chinense	P6890/56	JF949986	370.1	71.3	15.6	24	4	487.6	85.5	14.1	35	6
Saururaceae	*Houttuynia cordata*	P6875/41	JF950006	279.9	48.2	68.3	4	0.7	575.2	63.9	97.6	6	0.6
Schisandraceae	*Kadsura longipedunculata*	P6879/45	JF950007	9.9	1.8	0.1	99	18	86.1	43.9	11.8	7	3
Selaginellaceae	*Selaginella tamariscina*	P6904/70	JF950018	339.2	98.8	13.6	25	7	393.9	150.9	33.4	12	4
Valerianaceae	*Patrinia scabiosaefolia*	P6889/55	JF949985	140.5	38.7	13.7	10	3	159.4	15.9	19.0	8	0.8
Verbenaceae	*Verbena officinalis*	P6910/76	JF950020	298.1	145.9	16.5	18	9	334.7	37.7	20.5	16	1
Violaceae	*Viola yezoensis*	P6911/77	JF949993	59.6	60.8	3.3	18	18	297.5	19.1	24.7	12	0.7
Zingiberaceae	*Alpinia galanga*	P6837/03	-	55.7	5.7	1.4	39	4	111.7	53.4	15.4	7	3
	Alpinia oxyphylla	P6917/78	-	119.6	30.4	0.7	170	43	213.8	110.2	2.0	107	55

Quercetin 3-O-methyl ether and ophioglonin obtained from plants belonging to *Ophioglossaceae* genus have shown slight activity against HBV [50]. Since 1995 when antiviral activities against enveloped viruses were discovered in extracts of *Houttuynia cordata* [51], such as influenza, HIV, herpes, SARS and also in enteroviruses [51-54], 40 compounds have been isolated from the whole plant [55].

Among all of them, norcepharadione B has been identified as anti-herpes virus type 1 compound [55], quercetin may reduce virions production of HCV [56], but not against HBV [7] and quercetin 3-rhamnoside may be effective against influenza A virus [57].

Selaginella tamariscina has been a source of several drugs with anti-bacterial and antifungal activities such as amentoflavone [58], isocryptomerin [59-61], or with antitumor effects such as sterols [62] and biflavonoids [63]. *Alpinia galanga* crude extracts have been shown to have antibacterial activities [64] which seem to be enhanced in combination with other plants such as rosemary and lemon iron bark [65]. Compounds obtained from this plant, have also demonstrated other antimicrobial activities, such as anti-leishmanial phenylpropanoids [66] or 1'-acetoxychavicol acetate, and its halogenated derivatives (inhibitors of HIV-regulator protein Rev-export) [67-70].

The insecticidal properties of diarylheptanoid [71] as well as protective effects on anaphylactic reactions of the aqueous extracts from the fruit of *Alpinia oxyphylla* [72] have been described in the past. Recently anti-angiogenic properties of the fruit have been also discovered [73].

The water extracts were also screened against HBV in HepG2 2.2.15 cells (Table 3). *Evodia lepta*, *Hedyotis diffusa* and several *Glycyrrhiza* species lowered the HBV-DNA significantly and were not toxic to the HepG2 2.2.15 cell line (Figure 1).

Hardly anything is known about the other natural products of *Evodia lepta*, while the highly bioactive chromenes seem to be among the major constituents [74]. *Glycyrrhiza* species, on the other hand, are well known for their anti-inflammatory effects due to glycyrrhizic acid [9]. This genus is also known for its antiviral, especially antihepatitis properties [15,75]. Its ability to reduce the HBV-DNA in the culture medium of HepG2 2.2.15 at high doses has been previously reported [7]. *Heydiotis diffusa* again is a plant rich in iridoid glycosides with anti-inflammatory and hepatoprotective activities [76-78]. These compounds are most likely to be responsible for the effects against HBV.

Three enterohepatic cancer cell lines, HepG2 and SK-Hep1 (from human hepatoblastoma and hepatocarcinoma) and LS 174T (from human colon adenocarcinoma), were used to determine the antitumor ability of water extracts (Table 3). Twenty extracts were found to induce a significant antiproliferative effect with IC_{50} values between 0.5 and 20 μg/mL on these cell lines. These were further investigated to elucidate whether this was due to cytotoxicity.

In HepG2, none of the 4 extracts with ability to inhibit cell growth (*Coptis chinensis, Epimedium brevicornum, Equisetum hiemale* and *Senecio scandens*), were found to induce acute cell toxicity when they were incubated with the IC_{50} of the extracts for 6 h (Figure 2).

In SK-Hep1, among the 10 extracts with antitumor effect 7 did not induce acute toxicity (*Arctium lappa, Cassia tora, Centipeda minima, Chrysanthemum indicum, Coptis chinensis, Phellodendron chinense* and *Rheum palmatum*), whereas *Dysosma versipellis* was especially active by lowering the cell viability in comparison to the control to 40% (Figure 3). This is consistent with the inhibitory effects known for the lignans of *D. versipellis* against prostate cancer cell lines [79].

Figure 1. Effect of water extracts on hepatitis B virus (HBV) release as determined by HBV-DNA content in the culture medium and cell viability as determined by Neutral Red uptake by human hepatoblastoma cells HepG2 2.2.15 infected with HBV. Values are means ± SD of three experiments carried out in triplicate by incubation with the extracts for 21 days. *, $p < 0.05$ as compared with untreated cells by paired *t*-test.

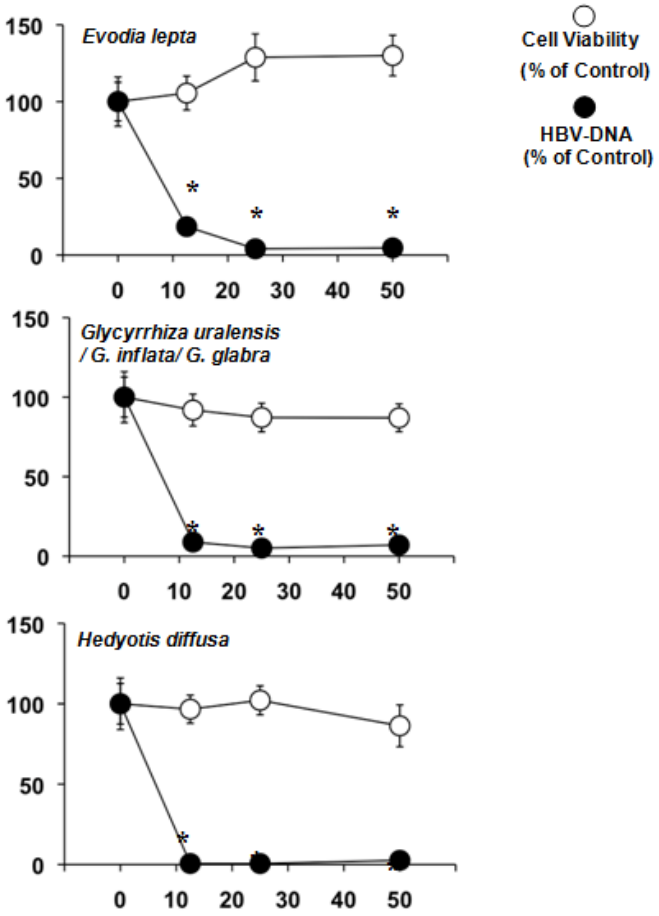


Figure 2. Acute cell toxicity as determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-difenyltetrazolium (MTT) assay in human hepatoblastoma HepG2 cells. Values are means ± SD of four experiments carried out in triplicate.

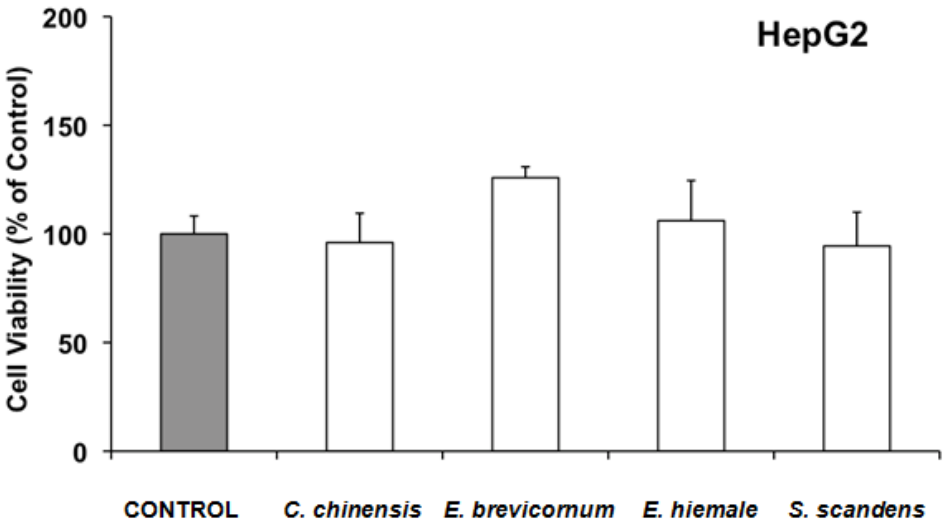
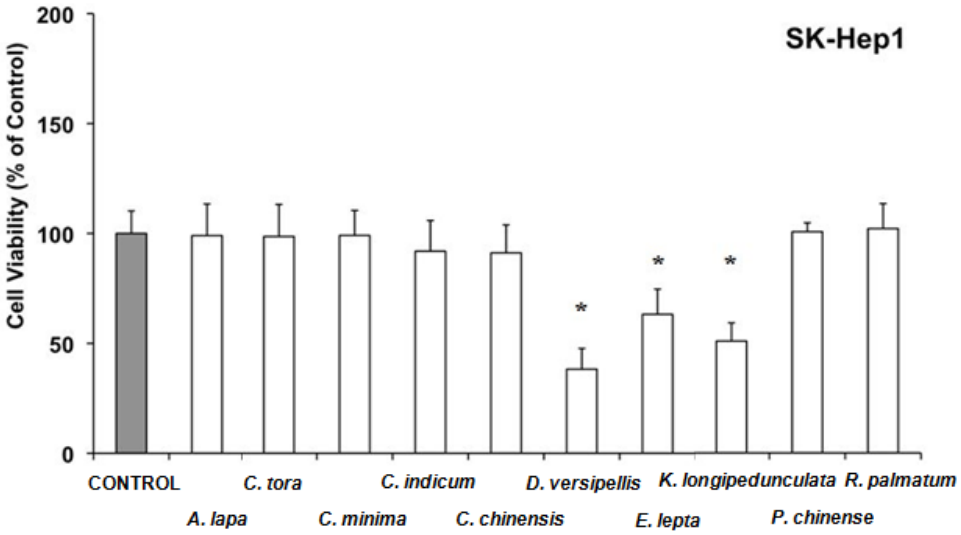
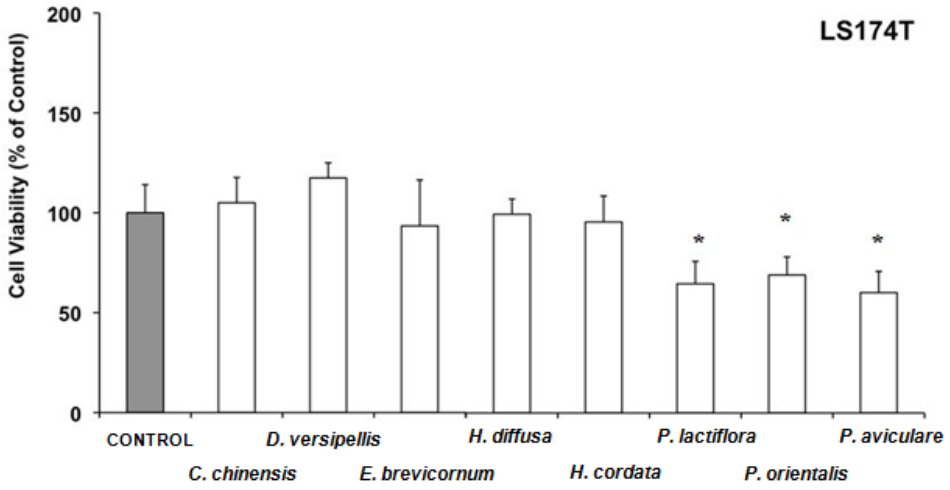


Figure 3. Acute cell toxicity as determined by MTT assay in human hepatoma SK-Hep1 cells. Values are means ± SD of four experiments carried out in triplicate. *, $p < 0.05$ as compared with Control by the Bonferroni method of multiple range testing.



Evodia lepta and *Kadsura longipedunculata* lowered also the cell viability of SK-Hep1 in comparison to the control to 50-60%. Recently, it has been reported that the essential oil of *Kadsura longipedunculata* and its major components (delta-cadinene, camphene, borneol, cubenol, and delta-cadinol) have some degree of cytotoxic activity against some human cell lines [80]. In LS 174T cells, water extracts from *Coptis chinensis, Dysosma versipellis, Epimedium brevicornum, Hedyotis diffusa* and *Houttuynia cordata* have antiproliferative effects without affecting cell viability (Figure 4), whereas *Paeonia lactiflora, Platycladus orientalis*, and *Polygonum aviculare*, in addition to inhibition of cell growth were able to acutely lower cell viability in comparison to the control to 60–70%.

Figure 4. Acute cell toxicity as determined by MTT assay in human colon adenocarcinoma LS 174T cells. Values are means ± SD of four experiments carried out in triplicate. *, $p < 0.05$ as compared with Control by the Bonferroni method of multiple range testing.



Paeonia lactiflora, which belongs to the Paeoniaceae family, is known as one of the richest sources of various resveratrol derivatives [81]. These phytoestrogens are known to exert strong antioxidant activity [81] and to inhibit growth of several cancer cell lines [82,83], including a colon human cell line [84]. Recently, the antiproliferative effects of essential oils obtained from *Platycladus orientalis* on human renal adenocarcinoma and amelanotic melanoma cells have been reported [85].

Coptis chinensis, which has been found active against the three enterohepatic cell lines, belongs to TCM formulations commonly used to treat liver diseases associated to infections by gastrointestinal parasite such as *Blastocystis hominis* [86]. Coptisine, which is used as gastric mucosa protector, and berberine, which has very interesting properties as antiinflamatory, antidiabetes, antidiarrhea, and hypocholesterolemic drug, have been obtained from this plant. Both of them have also shown antitumoral activities in *in vitro* models [87-92].

Animal cells offer several targets to natural products (Table 1). Of great importance are the biomembrane, the proteins and the DNA. Since human cells and trypanosomes share many similarities in the structure of the cells, it is extremely important to select those plant extracts where a great selectivity index (SI) occurred. The different cytotoxicity strongly hints to selective interactions between natural products and trypanosomes that do not occur in human cells. A great SI also hints to the relative absence or relative insignificance of general cytotoxic mechanisms like the unspecific interaction of phenolic OH-groups compared to more specific interactions with certain structures in trypanosomes.

The CH_2Cl_2 and MeOH extracts of 82 medicinal plants were screened against the cell lines HeLa, Cos7 and trypanosomes (*T. b. brucei*) (Table 4). The SI of the IC_{50} of mammalian cell/trypanosomes was regarded as significant if it was over 80. According to this criterium, seven extracts were highly selective towards trypanosomes.

The CH_2Cl_2 extract of *Alpinia oxyphylla* showed IC_{50} values of 119.6 μg/mL, 30.4 μg/mL and 0.7 μg/mL against HeLa, Cos7 and *T. b. brucei* respectively with SI of 170 and 43 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei* respectively. The MeOH extract of *A. oxyphylla* also was effective with IC_{50} values of 213.8 μg/mL, 110.2 μg/mL and 2.0 μg/mL against HeLa, Cos7 and *T. b. brucei* respectively. The SI was 107 and 55 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei* respectively. *A. oxyphylla* is basically an essential oil plant, so that we suspect the active principle to be based on the sesquiterpenes already known for their cytotoxic properties [93].

For *Kadsura longipedunculata* only the CH_2Cl_2 extract exhibited a significant selectivity. Here, the IC_{50} values of HeLa, Cos7 and *T. b. brucei* were 9.9 μg/mL, 1.8 μg/mL and 0.1 μg/mL respectively, resulting in SI of 99 and 18 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei* respectively. Essential oil and lignans form the major natural compounds of *K. longipedunculata* [80,94]. The specific trypanocidal effect rather seems to be based on the lignans than on the more unspecific essential oil. Further studies would be necessary to confirm this assumption.

For *Arctium lappa* only the CH_2Cl_2 extract showed a significant selectivity with IC_{50} values of 345.0 μg/mL, 344.2 μg/mL and 3.6 μg/mL against HeLa, Cos7 and *T. b. brucei* respectively and SI of 96 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei*.

In *Panax ginseng* and *P. notoginseng* the selectivity was again limited to the CH_2Cl_2 extract. *P. ginseng* gave IC_{50} values of 152.4 μg/mL, 47.7 μg/mL and 0.9 μg/mL against HeLa, Cos7 and *T. b. brucei* respectively with SI of 169 and 53 between HeLa and *T. b. brucei* and Cos7 and

T. b. brucei respectively. *P. notoginseng* demonstrated IC_{50} values of 263.0 μg/mL, 6.4 μg/mL and 0.9 μg/mL with SI of 292 and 7 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei* respectively.

Also an extremely active plant was *Saposhnikovia divaricata*. Here as well, lipophilic CH_2Cl_2 extract was selective with IC_{50} values of 410.1 μg/mL, 45.9 μg/mL and 5.1 μg/mL against HeLa, Cos7 and *T. b. brucei* respectively and SI of 80 and 9 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei* respectively.

The trypanocidal effects of *A. lappa, P. ginseng, P. notoginseng* and *S. divaricata* are based on the presence of highly reactive polyacetylenes, especially panaxynol.

Only the methanolic extract of *Coptis chinensis* showed a significant selectivity, but not the dichloromethane extract. The IC_{50} values of 81.8 μg/mL, 3.7 μg/mL and 0.4 μg/mL against HeLa, Cos7 and *T. b. brucei* respectively gave SI of 205 and 9 between HeLa and *T. b. brucei* and Cos7 and *T. b. brucei* respectively. Our analytical data confirmed berberine as the main alkaloid of *C. chinensis*. The toxicity of *C. chinensis* is probably an effect of the DNA intercalation of its alkaloids into the DNA double helix of *T. b. brucei* [95,96].

The trypanocidal effect of berberine against different trypanosoma species has been demonstrated previously. Merschjohann *et al.* [97] showed that *T. congolense* are sensitive to berberine at concentrations of 83 μM, while Rosenkranz and Wink [98] demonstrated a sensitivity of *T. brucei* to berberine at concentrations of only 0.5 μM. Recently, the effect of berberine against *T. rhodesiense* was also established by Freiburghaus *et al.* [99]. *T. rhodesiense* was sensitive to 4.2 μg/mL.

The significant differences in sensitivity of different trypanosoma species to berberine could be of high interest regarding resistance mechanisms against mutagenic compounds. Berberine might due to its mutagenic activity never become a lead structure for the development of trypanocidal drugs, but the differences in sensitivity of these three trypanosoma species might help to understand defence mechanisms against DNA intercalating substances.

4. Conclusions

Traditional Chinese and European Medicine comprise promising plants that might be used for antiviral, antitrypanosomal and anticancer therapy. The promising discoveries of highly effective plants against viral hepatitis, trypanosomiasis and liver and intestinal cancer cells, however, require further research to establish new lead structures or their combinations for the treatment of these important traditional diseases.

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Appendix

Origin and current area of use of the medicinal plants included in our study.

Family	Species	Origin	Area of Use
Acanthaceae	*Andrographis paniculata*	India	India, Sri Lanka, SE Asia, East Asia
Amaranthaceae	*Celosia cristata*	Tropical Asia	India, SE Asia, China, Africa, South America
Apiaceae	*Bupleurum chinense*	China	East Asia, China
	Bupleurum marginatum	China	East Asia, China
	Centella asiatica	East Asia, India, Sri Lanka, northern Australia, Iran, Melanesia, Papua New Guinea	East Asia, India, Sri Lanka, Australia, Melanesia, Papua New Guinea, Middle East, Africa
	Cnidium monnieri	China	East Asia, China
	Saposhnikovia divaricata	Central Asia (steppe region)	Central Asia, East Asia, China
Araliaceae	*Eleutherococcus senticosus*	Siberia	Siberia, China, Korea, Japan
	Panax ginseng China	China	Siberia, China, Korea, Japan
	Panax ginseng Korea	Korea	Siberia, China, Korea, Japan
	Panax notoginseng	China	Siberia, China, Korea, Japan
Arecaceae	*Areca catechu*	Malaysia, Philipines	SE Asia, East Asia, India, Sri Lanka, Papua New Guinea
Asclepiadaceae	*Cynanchum paniculatum*	SE Asia	East Asia, SE Asia
Asteraceae	*Artemisia annua*	Asia, introduced worldwide	worldwide
	Artemisia capillaris	Asia	Asia
	Arctium lappa	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia
	Centipeda minima	Asia, Himalaya	Asia
	Chrysanthemum indicum	India	Asia
	Chrysanthemum morifolium	Asia	Asia
	Eclipta prostrata	Tropical Asia, South America	Tropical Asia, East Asia, South America
	Senecio scandens	Asia	Asia
	Siegesbeckia orientalis	Tropical Asia	Tropical Asia, East Asia, Africa
	Taraxacum officinale	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
Berberidaceae	*Berberis bealei*	Asia, introduced in America, Europe	Asia, America, Europe
	Dysosma versipellis	East Asia	East Asia, China
	Epimedium koreanum	East Asia (Korea)	East Asia, China
Brassicaceae	*Capsella bursa-pastoris*	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia
	Isatis indigotica (root)	Central Asia (steppe region)	Central Asia, East Asia, China
	Isatis indigotica (leaf)	Central Asia (steppe region)	Central Asia, East Asia, China
Caprifoliaceae	*Lonicera confusa*	East Asia	East Asia, China
Convallariaceae	*Polygonatum kingianum*	Asia	Asia
Crassulaceae	*Rhodiola rosea*	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
Cupressaceae	*Platycladus orientalis*	China, introduced in most of Asia	Asia

Appendix. *Cont.*

Family	Species	Origin	Area of Use
Dryopteridaceae	*Cyrtomium fortunei*	Asia, introduced in America, Europe	Asia
Ephedraceae	*Ephedra sinica*	China	East Asia
Equisetaceae	*Equisetum hiemale*	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
Euphorbiaceae	*Croton tiglium*	SE Asia	East Asia, SE Asia
Fabaceae	*Abrus cantoniensis*	Southern China	East Asia, SE Asia
	Acacia catechu	East Asia, SE Asia	East Asia, SE Asia
	Cassia tora	East Asia, SE Asia, introduced to Middle and South America, Africa, Middle East	Europe, Asia, America, Africa
	Desmodium styracifolium	SE Asia	East Asia, SE Asia
	Glycyrrhiza inflata	Central Asia (Mongolia, China)	East Asia, Central Asia
	Spatholobus suberectus	Tropical Asia	India, East Asia, SE Asia
	Sutherlandia frutescens	South Africa	South Africa, Europe
Geraniaceae	*Geranium wilfordii*	East Asia	East Asia, China
	Pelargonium sidoides	South Africa	South Africa, Europe
Ginkgoaceae	*Ginkgo biloba*	China	Asia, Europe, North America
Hypericaceae	*Hypericum japonicum*	Japan	East Asia, China
Iridaceae	*Belamcanda chinensis*	China	East Asia, China
Lamiaceae	*Mentha haplocalyx*	China	East Asia, China
	Prunella vulgaris	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
	Scutellaria baicalensis	Central Asia (Russia, Mongolia, China)	East Asia, Central Asia
Lauraceae	*Cinnamomum cassia*	Tropical Asia (India, East Asia, SE Asia)	India, East Asia, SE Asia
Loranthaceae	*Taxillus chinensis*	China	East Asia, China
Lythraceae	*Punica granatum*	Middle East, Himalaya	Europe, Asia, America, Africa
Magnoliaceae	*Magnolia officinalis*	Himalaya, China	East Asia, China
Melanthiaceae	*Paris polyphylla*	Himalaya, China	East Asia, China
Myrsinaceae	*Lysimachia christinae*	China	East Asia, China
Myrtaceae	*Eucalyptus robusta*	East Australia	Europe, Asia, America, Africa, Australia
Ophioglossaceae	*Ophioglossum vulgatum*	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
Orchidaceae	*Dendrobium loddigesii*	SE Asia	East Asia, SE Asia
Paeoniaceae	*Paeonia lactiflora*	China	East Asia
Pedaliaceae	*Harpagophytum procumbens*	South Africa	South Africa, Europe
Poaceae	*Cymbopogon distans*	Himalaya, China	East Asia, China
Polygonaceae	*Fallopia japonica (syn. Polygonum cuspidatum)*	East Asia	East Asia, China
	Fallopia multiflora (syn. Polygonum multiflorum)	East Asia	East Asia, China
	Polygonum aviculare	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
	Rheum officinale	Asia	Europe, Asia, North America

Appendix. *Cont.*

Family	Species	Origin	Area of Use
Ranunculaceae	*Coptis chinensis*	China	East Asia, China
Rosaceae	*Rosa chinensis*	China	East Asia, China
	Rosa laevigata	SE Asia, China	Europe, Asia, North America
	Sanguisorba officinalis	Northern Hemisphere (Europe, Asia, North America)	Europe, Asia, North America
Rubiaceae	*Hedyotis diffusa*	East Asia	East Asia, China
Rutaceae	*Evodia lepta*	East Asia	East Asia, China
	Evodia rutaecarpa	East Asia	East Asia, China
	Phellodendron chinense	Himalaya, China	East Asia, China
Saururaceae	*Houttuynia cordata*	East Asia, SE Asia	East Asia, SE Asia
Schisandraceae	*Kadsura longipedunculata*	East Asia	East Asia, China
Selaginellaceae	*Selaginella tamariscina*	East Asia	East Asia, China
Valerianaceae	*Patrinia scabiosaefolia*	East Asia	East Asia, China
Verbenaceae	*Verbena officinalis*	Europe	Europe, Asia, North America
Violaceae	*Viola yezoensis*	East Asia	East Asia, China
Zingiberaceae	*Alpinia galanga*	SE Asia	East Asia, SE Asia
	Alpinia oxyphylla	SE Asia	East Asia, SE Asia