Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20160728031236

Original Research

ScopeMed

In vitro antileishmanial and antimalarial activity of selected plants of Nepal

Bishnu Joshi[1,2], Sarah Hendrickx[3], Lila Bahadur Magar[4], Niranjan Parajuli[4], Pierre Dorny[1], Louis Maes[3]

[1]Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, [2]Society for Biological Applications, Kathmandu, Nepal, [3]Department of Biomedical Sciences, Laboratory for Microbiology, Parasitology and Hygiene, University of Antwerp, Belgium, [4]Department of Biotechnology, National College, Tribhuvan University, Nayabazar, Kathmandu, Nepal

Address for correspondence:
Bishnu Joshi, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium. E-mail: joshi.bishnu@gmail.com

Received: May 15, 2016
Accepted: July 13, 2016
Published: August 14, 2016

ABSTRACT

Background: Nepal is very rich in biodiversity, and no extensive effort has yet been carried out to screen plants that are used by traditional healers against parasitic diseases. The aim of this study was to evaluate the *in vitro* antileishmanial and antimalarial activity of crude methanolic or ethanolic extracts of 29 plant species that are currently used by local people of Nepal for treating different ailments. **Methods:** Crude extracts of leaves, twigs, aerial parts, and/or roots of the selected plants were evaluated for *in vitro* inhibitory activity against intracellular amastigotes of *Leishmania infantum* and against erythrocytic stages of *Plasmodium falciparum*. To determine the selectivity index (SI), cytotoxicity was assessed on MRC-5 cells in parallel. **Results:** Three plant species, namely *Phragmites vallatoria* and *Ampelocissus tomentosa,* for which no antiprotozoal activity has previously been reported, and *Terminalia chebula* revealed antiprotozoal activity. The extract of *A. tomentosa* exhibited moderate activity against *L. infantum* with an inhibitory concentration 50% (IC_{50}) of 13.2 ± 4.3 μg/ml and SI >3, while *T. chebula* exhibited fairly good antiplasmodial activity with IC_{50} values of 4.5 ± 2.4 μg/ml and SI values >5. **Conclusion:** In countries like Nepal, where the current health system is unable to combat the burden of endemic parasitic diseases, evaluation of local plants as a potential source of the drug can help in expanding the treatment options. The extent of untapped resources available in these countries provides an opportunity for future bioprospecting.

KEY WORDS: Crude plant extracts, *in vitro, Leishmania,* Nepal, *Plasmodium*

INTRODUCTION

Leishmaniasis and malaria represent major public health problems with significant morbidity and mortality in Asia, Africa, and Latin America [1,2]. Lack of vaccines, emergence of drug resistance, and expensive chemotherapeutics are some of the major challenges for the control of these vector-borne diseases, in addition to disadvantages including hospitalization for (parenteral) treatment, occurrence of adverse effects, long-term therapy leading to poor compliance and poor availability of drugs, especially to economically weak populations residing in rural areas [3-6].

Given the limited number of novel drugs in the pipeline and the expanding resistance against current drugs, it remains imperative to explore alternative ways to find new drugs. Plants contain a broad diversity of secondary metabolites such as alkaloids, flavonoids, and phenolic derivatives that may have therapeutic value, and hence may represent an attractive source for novel drugs [7]. However, screening of each and every individual plant parts against wide range of pathogens is virtually impossible and plant selection based on ethnobotany and traditional practices, such as Ayurveda [8], Unani, Siddha, traditional Chinese medicine, and Japanese Kampo medicine increases the probability of finding "hit" molecules that can be subsequently developed toward "lead" development [9,10].

In Nepal, there is a huge variation in the number of medicinal and aromatic plants (MAP) [11,12]. For example, compilation of

the MAP database has listed 1624 medicinal plants in 2000 [13], rising to 1950 species in 2008 [14] clearly indicating that further exploration of the phytochemical and pharmacological properties of medicinal plants in Nepal should be continued. Up till now, very few indigenous Nepalese plants have been explored for their therapeutic potential against leishmaniasis and malaria. Starting from ethnobotanical literature and traditional use, the present study assessed the *in vitro* inhibitory activity potential of crude extracts of 29 selected Nepalese plants [Table 1], hence contributing to the medicinal knowledge of the local plant biodiversity.

MATERIALS AND METHODS

Plant Material

Leaves, twigs, aerial parts, and roots [Table 1] of selected plants were collected from different regions in Nepal [Figure 1] from December 2013 to April 2014. All the collected plant materials were identified in the Department of Plant Resources, Nepal, and Voucher specimens are deposited in Pharmacognosy Unit of Department of Plant Resources, Thapathali, Kathmandu, Nepal (http://www.dpr.gov.np).

Extraction

The plant materials were washed thoroughly with water and shade dried at room temperature. Dried samples were crushed into powder by electric blending and subjected to Soxhlet extraction using polar solvents (ethanol and methanol). The extracts were evaporated on a rotary evaporator under vacuum till a solid mass was obtained. The extracts were kept at 4°C until analysis. All the extracts were kept in sealed vials, labeled properly, and transported to the Laboratory of Microbiology Parasitology and Hygiene, University of Antwerp, for integrated *in vitro* screening.

Parasites and Cell Culture

Standard techniques **were used** as previously described [9]. Briefly, *ex vivo* amastigotes of *Leishmania infant*um (MHOM/MA(BE)/67) were used for the *in vitro* antileishmanial assay. The strain was routinely passed in Syrian Golden hamsters every 6-10 weeks. The chloroquine (CQ)-resistant Pl*asmodium falciparum* (K1 strain) was used for *in vitro* antiplasmodial activity testing. The human lung fibroblast cell line MRC-5 was cultured in minimum essential medium supplemented with 20 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5% fetal calf serum.

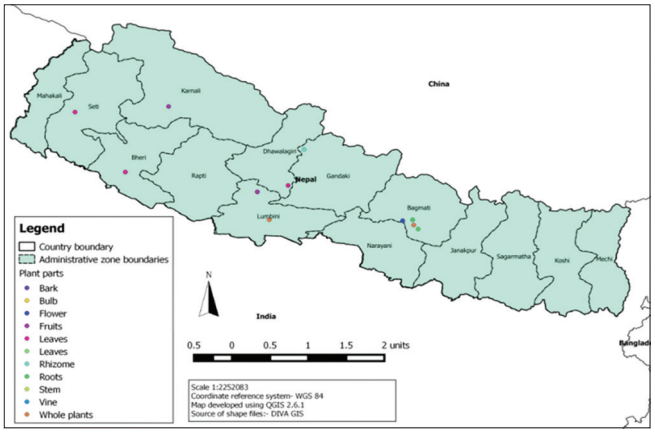

Figure 1: Sampling site in Nepal for the collection of plant species

Table 1: List of the selected plants for this study, their phytoconstituents, and traditional uses

Plant species	Family	Voucher specimen	Part	Constituents	Reported traditional use	Reference
Ageratum conyzoides (L.)	Asteraceae	NPRL_01	WP	Chromenes, benzofurans, flavonoids, farnesene derivatives daucanolides triterpenoids, sterols	In Asia, South America, and Africa, aqueous extract is used as a bactericide. It is also used to treat fever, rheumatism, and headache	[15-17]
Swertia chirayita (Roxb.)	Gentianaceae	NPRL_02	S, L	Xanthones, amarogentin	In Indian tradition - used in treating bronchial asthma, liver disorders, chronic fever, anemia, stomachache and diarrhea	[18-20]
Centella asiatica (L.) urb.	Apiaceae	NPRL_03	L	Centellin, centellicin, asiaticin	Used in the treatment of leprosy, skin disease and appetizer	[11]
Drymaria diandra Blume	Caryophyllaceae	NPRL_04	WP	Drymariatin A, diandrines A-D, flavonoids, alkaloiods	Root juice is inhaled to treat sinusitis	[21]
Syzygium aromaticum (L.)	Myrtaceae	NPRL_05	F, B	Eugenol, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid	Toothache and headache reliever, remedy against diarrhea, stomachache and bowel ailments, natural antihelminthic, antimalarial	[22]
Zanthoxylum armatum DC.	Rutaceae	NPRL_06	F, L	Alkaloids, flavonoids, coumarins	Prevent tooth decay, fruit and seed used in asthma and blood purifier. used for the treatment of malaria, GI disorders, gonorrhea	[23]
Cinnamomum zeylanicum Blume	Lauraceae	NPRL_07	B, L	Cinnamaldehyde, cinnamic acid, and cinnamate	Besides use as spice and flavoring agent, in Ayurvedic medicine - used for remedy for respiratory, digestive, and gynecological ailments	[24]

(Contd...)

Table 1: *(Continued)*

Plant species	Family	Voucher specimen	Part	Constituents	Reported traditional use	Reference
Cuminum cyminum L.	Apiaceae	NPRL_08	S, F	Cuminaldehyde, Safranal, Sesquiterpenes	Used in treatment of fever, loss of appetite, diarrhea, vomiting, abdominal distension, edema	[25]
Clerodendrum serratum Moon	Lamiaceae	NPRL_09	F, R	Triterpene, macrocyclic lactone, saponin, serratin, lupeol	Used to treat pain, inflammation, malaria fever in India	[26]
Ehretia acuminata R.Br.	Boranginaceae	NPRL_10	L	Methyl and ethyl esters	The leaves and branches are used in Chinese medicine	[27]
Oroxylum indicum (L.), benth. Ex. Kurtz	Bigoniaceae	NPRL_11	S, B, L	Chrysin, baicalein, baicalein-7-O-glucoside, baicalein-7-O-diglucoside	In treating Jaundice, diarrhea, malaria, arthritis, diabetes	[28]
Phragmites vallatoria (L.) Veldkamp	Poaceae	NPRL_12	WP	NA	Used in wound healing, arthritis, antiemetics, febrifuges, rheumatism, and diabetes	[29]
Pedilanthus tithymaloides (L.) Poit	Euphorbiaceae	NPRL_13	L	Anticancer-diterpene pedilstatin, octacosanol, cycloartenone and β-sitosterol	Traditionally used to heal wounds, burn, mouth ulcers, and venereal disease; found to be anticatarrhal, anti-inflammatory, antibiotic, antiseptic, antihemorrhagic, antiviral, antitumor	[30]
Kalanchoe pinnata Pers	Crassulaceae	NPRL_14	L, S, R	Bufadienolides, α- and β-amyrins	Antibacterial (respiratory tract infection), antiparasitic, antidepressant, anticancer, anti-insecticidal, antiallergic, anti-inflammatory, antidiabetes	[31]
Cirsium wallichii DC.	Asteraceae	NPRL_15	R	Thymol, β-linalool, eugenol	Used to treat fever, gastric problem, relieve burning sensation while urinating	[32]
Arisaema griffithii Schott.	Araceae	NPRL_16	L, R	NA	Used to treat malaria, eat as vegetable	[11]
Ampelocissus tomentosa (Roth) Planch.	Vitaceae	NPRL_17	F	NA	Used in menstrual bleeding, treating dysentery, fever, fistula, tuberculosis, and Insect bites	[33]
Arisaema griffithii Schott.	Araceae	NPRL_16	L, R	NA	Used to treat malaria, eat as vegetable	[11]
Dichrocephala integrifolia (L.f.) Kuntze.	Asteraceae	NPRL_18	L, F	Dichrocepholides A, B, C, parthenin	Used in treatment of malaria and hepatitis and wounds	[34]
Boenninghausenia albiflora (Hook.) Meisn.	Rutaceae	NPRL_19	R	Acridone, komalin, albiflorin-2 and albiflorin-3	Crushed plant placed inside nostrils in the treatment of malaria	[11,35]
Cynoglossum zeylanicum Thunb. ex. Lehm.	Boraginaceae	NPRL_20	WP	n-Hexadecanoic acid, stigmasterol, oleic acid	Decoction prepared from the whole plant is used to arrest vomiting	[27]
Sapindus mukorossi Gaertn.	Sapindaceae	NPRL_21	F	Triterpenoidal saponins	Used as expectorant, relieve joint pain, removing dandruff and the roots for treating gout and rheumatism	[36]
Anacyclus pyrethrum (L.) DC.	Asteraceae	NPRL_22	R	Phenolics, chlorogenic acid	In Ayurveda "*rasayana*"-Plant with immune modulators and also used in treatment of epilepsy and seizure	[37]
Adhatoda vasica Nees	Acanthaceae	NPRL_23	S	Alkaloids, tannins, saponins	Used to treat bronchitis, relieves cough and breathlessness, stop bleedings	[38]
Boerhavia diffusa L.	Nyctaginaceae	NPRL_25	R	Boeravinones, Rotenoids	Used in jaundice, kidney problems, skin troubles, eye diseases, wounds, and inflammation	[39]
Terminalia chebula Retz.	Combretaceae	NPRL_26	Fr	Chebulanin, punicalagin, terchebin, gallic acid, flavonoids, ursolic acid	Used in treatment of asthma, sore throat, vomiting, hiccough, diarrhea, dysentery, bleeding piles, ulcers, gout	[40]
Rhododendron arboreum Sm.	Ericaceae	NPRL_27	F, L	Quercitrin, and coumaric acid	Used in vomiting, cough menstrual disorder, headache, throat ache, rheumatic pain	[41]
Paris pollyphylla Sm.	Trilliaceae	NPRL_28	Rh	Diosgenin and pennogenin saponin	Gastric and menstrual problem, to remove worms	[42]
Aleuritopteris anceps (Blanf.) panigrahi	Pteridaceae	NPRL_29	L, S	Chalcones, flavonols, flavonol-esters, kaempferol and quercetin	Used in preventing infection and inflammation	[43]
Parthenium hysterophorus L.	Asteraceae	NPRL_30	WP	Sesquiterpene lactones, caffeic acid, chlorogenic acid, ferulic acid, sitosterol	Used to treat fever, diarrhea, neurologic disorders, UTI dysentery, and malaria	[44]

L: Leaves, S: Stem, R: Root, B: Bark, F: Flower, WP: Whole plant, Rh: Rhizome, Fr: Fruit, NA: Not available, GI: Gastrointestinal

Biological *In Vitro* Assays

The integrated panel of microbial screens and standard screening methodologies were adopted as previously described [9]. Plant extracts were tested at dilutions ranging from 128 to 0.25 μg/mL using automated robotics with a 10-fold serial dilution strategy. Initially, 2-fold serial dilutions were made in 100% dimethyl sulfoxide (DMSO) to ascertain complete solubility during the dilution process. An immediate dilution step was performed in Milli-Q water before transferring the respective compound dilutions to the test plates (1/20 dilution: 10 μL compound solution +190 μL cell medium and test system) so that the final in-test concentration of DMSO did not exceed 1%.

Antileishmanial Activity

Mouse macrophages were stimulated by intraperitoneal injection of starch. 2 days after injection, macrophages were collected and seeded in each well (3×10^4) of a 96-well plate. The plates were incubated at 37°C and 5% CO_2. After 2 days of outgrowth, *ex vivo* amastigotes were used to infect primary peritoneal mouse macrophages at a 10:1 infection ratio. The plates were further incubated for 2 h before the compound dilutions were added. After 5 days of incubation, cells were dried, fixed with methanol, and stained with 20% Giemsa to assess total intracellular amastigote burdens through microscopic reading. The results are expressed as the percentage reduction of amastigote burden compared to untreated control cultures and inhibitory concentration 50% (IC_{50})-values were calculated.

Antiplasmodial Assay

CQ-resistant *P. falciparum* 2/K 1-strain was cultured in human erythrocytes O^+ at 37°C under microaerophilic atmosphere (3% O_2, 4% CO_2, and 93% N_2) in RPMI-1640 supplemented with 10% human serum. 200 μL of infected red blood cells (1% parasitemia and 2% hematocrit) was added in each well of a 96 well plate containing prediluted extract. The test plates were kept in the modular incubator chamber for 72 h at 37°C, and subsequently, put at −20°C to lyse the red cells upon thawing. Next, 100 μL of Malstat™ reagent was put in new microtiter plate to which 20 μL of hemolyzed parasite suspension was added. After 15 min incubation at room temperature, 20 μl of nitro blue tetrazolium/polyethersulfone solution was added. The plate was incubated in the dark for another 2 h at room temperature and spectrophotometrically read at 655 nm. The IC_{50} was calculated from the drug concentration - response curves. According to the WHO guidelines ([45]), antiplasmodial activity is very good with IC_{50} <1 μg/ml; good to moderate if IC_{50} of 1-10 μg/ml; weak if 15-50 μg/ml, and inactive if IC_{50} >50 μg/ml, always taking into account a selectivity index (SI) higher than 10.

RESULTS

Antileishmanial Activity

Only one plant extract (*Ampelocissus tomentosa)* exhibited moderate activity against *L. infantum* with an IC_{50} value of 13.2 ± 4.3 μg/ml and an SI value >3. *Paris polyphylla* also showed inhibitory activity but was also cytotoxic [Table 2].

Antiplasmodial Activity

Three plant species, *Phragmites vallatoria*, *A. tomentosa*, and *Terminalia chebula* showed schizonticidal activity. Among them, *T. chebula* exhibited the best activity with IC_{50} values of 4.5 ± 2.4 μg/ml and SI values >5.

Cytotoxicity

Kalanchoe pinnata, *P. polyphylla*, and *Pedilanthus tithymaloides* were toxic to the MRC-5 cell line. *K. pinnata* was most toxic with cytotoxic concentration 50% value of 4.7 ± 1.8 μg/ml.

DISCUSSION AND CONCLUSION

Leishmaniasis and malaria continue to be major public health problems, and the available drugs are generally expensive and not devoid of toxic side effects. Associated with poor compliance, the threat of drug resistance is also an emerging issue. Despite different strategies such as drug repurposing, identifying new therapeutic targets by chemoinformatics or screening diverse libraries of natural products, no new drugs have reached the market during the last decade. The present study was carried out to explore the potential of Nepalese medicinal plants that are used as part of traditional medicine. Nepal is very rich in biodiversity, which has not yet been explored satisfactorily due to the geopolitical situation, the lack of sophisticated labs, and the availability of trained manpower in industry and academics. The selected medicinal plants were screened against protozoal diseases using a "whole-cell based" approach, which can be considered more valid than enzyme-based subcellular approaches [9].

In the present study, *A. tomentosa* showed selective antileishmanial (IC_{50} 13.2 ± 4.3 μg/ml) and antimalarial (11.7 ± 3.5 μg/ml) activity. To our knowledge, the antiprotozoal activity of this plant has never been investigated, and no active constituents have been documented in the literature. Further studies on bioassay-guided fractionation to identify the putative active constituents and to better understand the therapeutic targets will be necessary, including a screening of other species of *Ampelocissus* genus.

Likewise, good antimalarial activity was found for *T. chebula* and *P. vallatoria* with an IC_{50} of 4.5 ± 2.4 and 12.0 ± 7.5, respectively, and SI of >5. This is the first observation that *P. vallatoria* showed potential activity against *Plasmodium*. The antiplasmodial activity of *T. chebula* has already been reported [22] with an IC_{50} = 4.76 μg/mL against the CQ-sensitive (3D7) strain of *P. falciparum*, hence supporting its use in traditional medicine.

P. tithymaloides was also found to be active against *Leishmania* but was not totally devoid of cytotoxicity. In traditional medicine, *P. tithymaloides* is been used in treating multiple diseases (from antimicrobial to anticancer) related to the

Table 2: Antiprotozoal activity of extract of selected plants of Nepal and their cytotoxicity against MRC-5 cell lines

Plant	Family	Solvent	Part used	*L. infantum*	SI	*P. falciparum*		MRC-5
				IC_{50} (μg/ml)		IC_{50} (μg/ml)	SI	CC_{50}
Ageratum conyzoides	Asteraceae	Ethanol	WP	96.5	0.6	72.4±28.3	0.8	62.7±3.3
Swertia chirayita	Gentianaceae	Ethanol	L	>128	nd	>128	nd	>128
Centella asiatica	Apiaceae	Ethanol	L	>128	nd	>128	nd	>128
Drymaria diandra	Caryophyllaceae	Ethanol	WP	>128	nd	>128	nd	>128
Syzygium aromaticum	Myrtaceae	Ethanol	L	61.5±5.9	1.0	17.8±2.9	3.60	64.4±7.4
Zanthoxylum armatum	Rutaceae	Ethanol	L	18.8±3.7	3.1	23.5±1.5	2.7	63.8±2.5
Cinnamomum zeylanicum	Lauraceae	Ethanol	L	48.1	0.9	42.8	1.1	47.1
Cuminum cyminum	Apiaceae	Ethanol	Fr	64.4	1.3	18.8	4.3	81.7
Clerodendrum serratum	Verbenaceae	Methanol	R	65.4±11.1	0.4	65.4±11.1	0.3	25.3±6.8
Ehretia acuminata	Boraginaceae	Methanol	L	54.5	0.9	12.1	4.2	50.5
Oroxylum indicum	Bignoniaceae	Methanol	B	52.7±17.1	nd	>128	nd	>128
Phragmites vallatoria	Poaceae	Methanol	WP	>128	nd	12.0±7.5	>5	63.9±1.4
Pedilanthus tithymaloides	Euphorbiaceae	Methanol	S	11.8±2.4	1.1	30.6±1.9	0.4	12.8±2.3
Kalanchoe pinnata	Crassulaceae	Methanol	L	44.6±21.6	0.1	>128	nd	4.7±1.8
Cirsium wallichii	Asteraceae	Methanol	R	>128	nd	101.6	nd	>128
Arisaema griffithii	Araceae	Methanol	B	>128	nd	>128	nd	>128
Ampelocissus tomentosa	Vitaceae	Methanol	V	13.2±4.3	3.5	11.7±3.5	4.1	47.1±6.1
Dichrocephala integrifolia	Asteraceae	Methanol	L	64.9	1.0	52.2±13.4	1.2	65.5±0.2
Boenninghausenia albiflora	Rutaceae	Methanol	L	55.8	0.3	32.9±15.5	0.5	16.8±7.1
Cynoglossum zeylanicum	Boraginaceae	Methanol	L	64.9	0.3	79.6±4.5	0.6	49.1±16.5
Sapindus mukorossi	Sapindaceae	Methanol	L	63.4	0.4	47.5±0.6	0.4	23.7±3.2
Anacyclus pyrethrum	Asteraceae	Methanol	Rh	86.1	nd	52.8	nd	>128
Adhatoda vasica	Acanthaceae	Methanol	L	64.9	nd	40.5	nd	>128
Boerhavia diffusa	Nyctaginaceae	Methanol	WP	52.8	1.0	37.6±12.8	1.4	54.6±6.1
Terminalia chebula	Combretacea	Methanol	Fr	64.9	0.5	4.5±2.4	>5	35.6±1.9
Rhododendron arboreum	Ericaceae	Ethanol	F	64	nd	42.9	nd	>128
Paris polyphylla	Trilliaceae	Ethanol	Rh	8.8±6.7	1.4	>128	nd	13.3±0.8
Aleuritopteris anceps	Pteridaceae	Methanol	L	101.6	0.8	48.7	1.7	85.5
Parthenium hysterophorus	Asteraceae	Methanol	WP	64.6	1.3	47.1	1.8	50.8

L: Leaves, S: Stem, R: Root, B: Bark, F: Flower, Fr: Fruit, WP: Whole plant, Rh: Rhizome, nd: Not determined, *L. infantum: Leishmania infantum, P. falciparum: Plasmodium falciparum,* SI: Selectivity index, IC_{50}: Inhibitory concentration 50%, CC_{50}: Cytotoxic concentration 50%

diverse phytoconstituents [Table 1]. The antiprotozoal activity of this plant might be due to the presence of a diterpene, as species belonging to the family Euphorbiaceae are rich in diterpenoids and triterpenoids [46]. In previous studies, various poly-O-acylated jatrophane diterpenoids have shown *in vitro* antiplasmodial activity with IC_{50} values of 3.4-4.4 μg/ml, which has been confirmed *in vivo*, with 76% suppression of parasitemia in *P. berghei* infected mice [47,48]. Likewise, diterpenes such as jatrogrossidione and jatrophone have been found to have toxic effects against promastigotes of *L. braziliensis*, *L. amazonensis*, and *L. chagasi* with IC_{50} in the range of 0.75-5 μg/ml [49]. The moderate cytotoxic nature of *P. tithymaloides* might be due to the presence of pedilstatin or eurphorbol, which have already been established as irritants and carcinogens [50].

Non-selective antileishmanial activity was shown for *P. polyphylla* and *K. pinnata*. *P. polyphylla* is known as "*satuwa*" and is traditionally used as anthelmintic and for reducing fever in the Himalayan region of Nepal. Our findings on cell toxicity of some plant extracts (IC_{50} 15 μg/ml) warrants for some vigilance as sometimes misleading information like "natural products are always safe" could eventually lead to deleterious health if high doses of these plants are consumed for a long time. Quite a lot of published literature indeed lacks parallel cytotoxicity evaluation. For example, *P. polyphylla* diosgenin-type saponins revealed antileishmanial activity (IC_{50} 1.6 μg/ml) but without parallel cytotoxicity evaluation [42]. In our study, *K. pinnata* was highly cytotoxic (4.7 ± 1.8 μg/ml) while published data support that *K. pinnata* may possess immunosuppressive effects and inhibit disease progression in *L. amazonensis*-infected individuals [31,51,52]. The same research group more recently reported that this plant possessed immunomodulatory activity and highlighted that oral dose of *K. pinnata* extract (400 mg/kg) is comparable to

Pentostam® (72 mg/kg) in reducing the hepatic and splenic parasitic burden [53].

Further research on these plants should now focus on the structural elucidation of the putative "active constituents," *in vitro* evaluation using preset IC_{50} and SI cut-offs and *in vivo* evaluation in murine pharmacology models for pharmacokinetic and dynamic profiling.

ACKNOWLEDGMENTS

We are very much thankful to Dr. Jyoti Joshi Bhatta (Department of Plant Resources), Sunil Lekhak and Keshab Ranabhat for arranging plant sample collection and shipment to Belgium. We are also thankful to staffs of University of Antwerp (An Matheeussen, Natascha Van Pelt and Pim-Bart Feijens) for their generous help in *in vitro* screening work.

REFERENCES

1. Trouiller P, Olliaro P, Torreele E, Orbinski J, Laing R, Ford N. Drug development for neglected diseases: A deficient market and a public-health policy failure. Lancet 2002;359:2188-94.
2. Andrews KT, Fisher G, Skinner-Adams TS. Drug repurposing and human parasitic protozoan diseases. Int J Parasitol Drugs Drug Resist 2014;4:95-111.
3. Croft SL, Yardley V. Chemotherapy of leishmaniasis. Curr Pharm Des 2002;8:319-42.
4. Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BP, Nakamura CV. Recent advances in leishmaniasis treatment. Int J Infect Dis 2011;15:e525-32.
5. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, *et al*. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 2009;361:455-67.
6. Croft SL, Olliaro P. Leishmaniasis chemotherapy--challenges and opportunities. Clin Microbiol Infect 2011;17:1478-83.
7. Wink M. Medicinal plants: A source of anti - Parasitic secondary metabolites. Molecules 2012;17:12771-91.
8. Kunwar RM, Bussmann RW. Ethnobotany in the Nepal Himalaya. J Ethnobiol Ethnomed 2008;4:24.
9. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. J Ethnopharmacol 2006;106:290-302.
10. Patwardhan B, Mashelkar RA. Traditional medicine-inspired approaches to drug discovery: Can Ayurveda show the way forward? Drug Discov Today 2009;14:804-11.
11. Manandhar NP. Plants and People of Nepal. Portland, OR: Timber Press; 2002.
12. Baral SR, Kurmi PP. Compendium of Medicinal Plants in Nepal. Chabahil: Rachana Sharma; 2006.
13. Shrestha K, Tiwari N, Ghimire S. Medicinal and aromatic plants database of Nepal (MAPDON). In: Proceedings of Nepal-Japan Joint Symposium on Conservation and Utilization of Himalayan Medicinal Plant Resources. 2000. p. 53-74.
14. Ghimire S. Medicinal plants in the Nepal Himalaya: Current issues, sustainable harvesting, knowledge gaps and research priorities. In: Medicinal Plants in Nepal: An Analogy of Contemporary Research. Nepal: Ecological Society; 2008. p. 25-44.
15. Ming LC. Ageratum conyzoides: A tropical source of medicinal and agricultural products. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press; 1999. p. 469-73.
16. Oladejo OW, Imosemi IO, Osuagwu FC, Oyedele OO, Oluwadara OO, Ekpo OE, *et al*. A comparative study of the wound healing properties of honey and *Ageratum conyzoides*. Afr J Med Med Sci 2003;32:193-6.
17. Nour AM, Khalid SA, Kaiser M, Brun R, Abdalla WE, Schmidt TJ. The antiprotozoal activity of methylated flavonoids from *Ageratum conyzoides* L. J Ethnopharmacol 2010;129:127-30.
18. Brahmachari G, Mondal S, Gangopadhyay A, Gorai D, Mukhopadhyay B, Saha S, *et al*. Swertia (Gentianaceae): Chemical and pharmacological aspects. Chem Biodivers 2004;1:1627-51.
19. Chen Y, Huang B, He J, Han L, Zhan Y, Wang Y. *In vitro* and *in vivo* antioxidant effects of the ethanolic extract of *Swertia chirayita*. J Ethnopharmacol 2011;136:309-15.
20. Laxmi A, Siddhartha S, Archana M. Antimicrobial screening of methanol and aqueous extracts of *Swertia chirata*. Int J Pharm Pharma Sci 2011;3 Suppl 4:142-6.
21. Singh AG, Kumar A, Tewari DD. An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal. J Ethnobiol Ethnomed 2012;8:19.
22. Bagavan A, Rahuman AA, Kaushik NK, Sahal D. *In vitro* antimalarial activity of medicinal plant extracts against *Plasmodium falciparum*. Parasitol Res 2011;108:15-22.
23. Bhatia H, Sharma YP, Manhas RK, Kumar K. Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India. J Ethnopharmacol 2014;151:1005-18.
24. Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. Evid Based Complement Alternat Med 2014;2014:642942.
25. Johri RK. *Cuminum cyminum* and *Carum carvi:* An update. Pharmacogn Rev 2011;5:63-72.
26. Patel JJ, Acharya SR, Acharya NS. *Clerodendrum serratum* (L.) Moon. - A review on traditional uses, phytochemistry and pharmacological activities. J Ethnopharmacol 2014;154:268-85.
27. Manandhar N. Traditional medicinal plants used by tribals of Lamjung district, Nepal. Pharm Biol 1987;25:236-40.
28. Dinda B, SilSarma I, Dinda M, Rudrapaul P. *Oroxylum indicum* (L.) Kurz, an important Asian traditional medicine: From traditional uses to scientific data for its commercial exploitation. J Ethnopharmacol 2015;161:255-78.
29. Krishna A, Saradhi MP, Rao BN, Mohan P, Balaji M, Samuel L, *et al*. Evaluation of wound healing activity of *Phragmites vallatoria* leaf ethanol extract in stz-induced diabetic rats. Int J Pharm Pharm Sci 2012;4:393-5.
30. Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. J Ethnopharmacol 2013;145:554-65.
31. Muzitano MF, Tinoco LW, Guette C, Kaiser CR, Rossi-Bergmann B, Costa SS. The antileishmanial activity assessment of unusual flavonoids from *Kalanchoe pinnata*. Phytochemistry 2006;67:2071-7.
32. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J Ethnobiol Ethnomed 2006;2:14.
33. Srivastava A, Patel SP, Mishra RK, Vashistha RK, Singh A, Puskar AK. Ethnomedicinal importance of the plants of Amarkantak region, Madhya Pradesh, India. Int J Med Aromatic Plants 2012;2:53-9.
34. Morikawa T, Abdel-Halim OB, Matsuda H, Ando S, Muraoka O, Yoshikawa M. Pseudoguaiane - Type sesquiterpenes and inhibitors on nitric oxide production from *Dichrocephala integrifolia*. Tetrahedron 2006;62:6435-42.
35. Chaya N, Terauchi K, Yamagata Y, Kinjo J, Okabe H. Antiproliferative constituents in plants 14. Coumarins and acridone alkaloids from *Boenninghausenia japonica* NAKAI. Biol Pharm Bull 2004;27:1312-6.
36. Kunwar RM, Mahat L, Acharya RP, Bussmann RW. Medicinal plants, traditional medicine, markets and management in far-west Nepal. J Ethnobiol Ethnomed 2013;9:24.
37. Kushwaha MN, Jatav VS, Pandey S. Plant *Anacyclus pyrethrum* - A review. Res J Pharm Phytochemistry 2012;4:164-70.
38. Venkatesalu V, Gopalan N, Pillai CR, Singh V, Chandrasekaran M, Senthilkumar A, *et al*. *In vitro* anti-plasmodial activity of some traditionally used medicinal plants against *Plasmodium falciparum*. Parasitol Res 2012;111:497-501.
39. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. Biomed Res Int 2014;2014:808302.
40. Bag A, Bhattacharyya SK, Chattopadhyay RR. The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. Asian Pac J Trop Biomed 2013;3:244-52.
41. Coburn B. Some native medicinal plants of the western Gurung. Kailash 1984;11:55-88.
42. Devkota KP, Khan MT, Ranjit R, Lannang AM, Samreen, Choudhary MI. Tyrosinase inhibitory and antileishmanial constituents from the rhizomes of *Paris polyphylla*. Nat Prod Res 2007;21:321-7.

43. Chowdhary S, Verma D, Pande R, Kumarc H. Antioxidative properties of flavonoids from *Cheilanthes anceps* Swartz. J Am Sci 2010;6:22-6.
44. Patel S. Harmful and beneficial aspects of *Parthenium hysterophorus*: An update. 3 Biotech 2011;1:1-9.
45. Ezzat SM, Salama MM, Mahrous EA, Maes L, Pan CH, Abdel-Sattar E. Antiprotozoal activity of major constituents from the bioactive fraction of *Verbesina encelioides*. Nat Prod Res 2016:1-5.
46. de Carvalho PB, Ferreira EI. Leishmaniasis phytotherapy. Nature's leadership against an ancient disease. Fitoterapia 2001;72:599-618.
47. Mongkolvisut W, Sutthivaiyakit S. Antimalarial and antituberculous poly-O-acylated jatrophane diterpenoids from *Pedilanthus tithymaloides*. J Nat Prod 2007;70:1434-8.
48. Adzu B, Zakariya S, Auta I, Katsayal U. Assessing the potency of *Pedilanthus tithymaloides* latex against *Plasmodium berghei* berghei infected mice. Int J Biol and Chem Sci 2008;2:216-9.
49. Sauvain M, Moretti C, Muiioz V, Ruiz E, Balanza E. Jatropha grossidentata and jatrophone from *Jatropha isabellii*. Phytother Res 1996;10:378.
50. Pettit GR, Ducki S, Tan R, Gardella RS, McMahon JB, Boyd MR, *et al*. Isolation and structure of pedilstatin from a republic of maldives Pedilanthus sp. J Nat Prod 2002;65:1262-5.
51. Rossi-Bergmann B, Costa S, Borges M, Da Silva S, Noleto G, Souza M, Moraes V. Immunosuppressive effect of the aqueous extract of *Kalanchoe pinnata* in mice. Phytother Res 1994;8:399-402.
52. Da Silva SA, Costa SS, Mendonca SC, Silva EM, Moraes VL, Rossi-Bergmann B. Therapeutic effect of oral *Kalanchoe pinnata* leaf extract in murine leishmaniasis. Acta Trop 1995;60:201-10.
53. Gomes DC, Muzitano MF, Costa SS, Rossi-Bergmann B. Effectiveness of the immunomodulatory extract of *Kalanchoe pinnata* against murine visceral leishmaniasis. Parasitology 2010;137:613-8.

Source of Support: Nil, **Conflict of Interest:** None declared.