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 part 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

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% (IC50) of 13.2 ± 4.3 μg/ml and SI >3, while T. chebula exhibited fairly good antiplasmodial activity with IC50 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
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 infantum (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 Plasmodium 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 NaHCO₃, and 5% fetal calf serum.

| 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 Xanthones, amarogentin | 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                                              | Root juice is inhaled to treat sinusitis                                                   | [11]      |
| Drymaria diandra Blume   | Caryophyllaceae | NPRL_04         | WP   | Drymariaitin A, diadromes A-D, flavonoids, alkaloids                           |                                                                                           | [21]      |
| Syzygium aromaticum (L.) | Myrtaceae   | NPRL_05          | F, B | Eugenol, biflorin, kaempferol, rhhamnositin, myricetin, gallic acid            | Toothache and headache reliever, remedy against diarrhea, stomachache and bowel ailments, natural anthelmintic, 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 disturbances, 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]      |

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

Figure 1: Sampling site in Nepal for the collection of plant species
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. | Boraginaceae | 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 | Chrysos, baicalein, baicalein-7-O-glucoisde, baicalein-7-O-diglucoside | In treating Jaundice, diarreha, 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 anticitarrhal, anti-inflammatory, antibiotic, antiseptic, antihemorrhagic, antiviral, antitumor | [30] |
| Kalanchoe pinnata Pers | Crassulaceae | NPRL_14 | L, S, R | Bufadienolides, α- and β-amymins | Antibacterial (respiratory tract infection), antiparasitic, antidepressant, anticancer, anti-insecticidal, antiallergic, anti-inflammatory, anti-diabetes | [31] |
| Cirsiun wallichii DC. | Asteraceae | NPRL_15 | R | Thymol, β-linalool, eugenol | Used to treat fever, gastric problem, relief 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] |
| Anacystis pyrethrum (L.) DC. | Asteraceae | NPRL_18 | L, F | Dichocepholides A, B, C, parthenin | Used in treatment of malaria and hepatitis and wounds | [34] |
| Dichrocephalina integrifolia (L.f.) Kuntze | Asteraceae | NPRL_19 | R | Acidrone, komalin, albiflorin-2 and albiflorin-3 | Crushed plant placed inside nostrils in the treatment of malaria | [11,35] |
| Boenninghausenia albigflora (Hook.) Meisn. | Rutaceae | NPRL_20 | WP | n-Hexadecanoic acid, stigmasterol, oleic acid | Decoction prepared from the whole plant is used to arrest vomiting | [27] |
| Cynoglossum zeylanicum Thunb. ex. Lehm. | Boraginaceae | NPRL_21 | F | Triterpenoid saponins | Used as expectorant, relieve joint pain, removing dandruff and the roots for treating gout and rheumatism | [36] |
| Sapindus mukorossi Gaertn. | Sapindaceae | NPRL_22 | F | Triterpenoidal saponins | In Ayurveda “rasayana”-Plant with immune modulators and also used in treatment of epilepsy and seizure | [37] |
| Anacyclus pyrethrum (L.) DC. | Asteraceae | NPRL_23 | S | Alkaloids, tannins, saponins | Used to treat bronchitis, relieves cough and breathingness, stop bleedings | [38] |
| Adhatoda vasica Nees | Acanthaceae | NPRL_24 | S | Boeravinones, Rotenoids | Used in jaundice, kidney problems, skin troubles, eye diseases, wounds, and inflammation | [39] |
| Terminalia chebulan Retz. Combretaceae | NPRL_25 | Fr | Chebulanin, punicalagin, terchebin, gallic acid, flavonoids, usrisol acid | Used in treatment of asthma, sore throat, vomiting, hiccough, diarrhea, dysentery, bleeding piles, ulcers, gout | [40] |
| Rhododendron arboreum Sm. | Ericaceae | NPRL_26 | F, L | Quercitrin, and coumaric acid | Used in vomiting, cough menstrual disorder, headache, throat ache, rheumatic pain | [41] |
| Paris polyphylla Sm. | Trilliaceae | NPRL_27 | Rh | Diosgenin and penogenin saponin | Gastric and menstrual problem, to remove worms | [42] |
| Aleuriteophuris aniceps (Blanf.) panigrahi | Pteridaceae | NPRL_28 | L, S | Chalcolines, flavonols, flavonol-esters, kaempferol and quercetin | Used in preventing infection and inflammation | [43] |
| Parthenium hysterophorus L. | Asteraceae | NPRL_30 | WP | Sesquiterpene lactone, 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 × 10⁴) of a 96-well plate. The plates were incubated at 37°C and 5% CO₂. 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 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% (IC₅₀)-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% O₂, 4% CO₂, and 93% N₂) 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, plates were further incubated for 2 h before the compound dilutions of DMSO did not exceed 1%.

RESULTS

Antileishmanial Activity

Only one plant extract (Ampelocissus tomentosa) exhibited moderate activity against L. infantum with an IC₅₀ 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 IC₅₀ values of 4.5 ± 2.4 μg/ml and SI values >5.

Cytotoxicity

Kalanche 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 (IC₅₀ 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 IC₅₀ 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 IC₅₀ = 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
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 IC50 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 IC50 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 “satwa” 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 (IC50 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 (IC50 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

### 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 IC50 (μg/ml) | P. falciparum IC50 (μg/ml) | MRC-5 CC50 |
|----------------------|-----------------|---------|-----------|--------------------------|----------------------------|------------|
| 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    | Boragineae      | Methanol | L         | 54.5 0.9                  | 12.1 4.2                    | 50.5       |
| Oroxyllum indicum    | Bignoniaceae    | Methanol | B         | 52.7±17.1 nd             | >128 nd                    | >128       |
| Phragmites vallatoria| Poaceae         | Methanol | WP        | >128 nd                  | 12.0±7.5 5.0               | 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   |
| Dicrocephalia 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 | Boragineae | 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   |
| Anacystis 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   | Combretaceae    | Methanol | Fr        | 64.9 0.5                  | 4.5±2.4 5.0               | 35.6±1.9   |
| Rhododendron arboreum| Ericaceae       | Methanol | 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 aniceps | 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, IC50: Inhibitory concentration 50%, CC50: Cytotoxic concentration 50%.
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 IC₅₀ and SI cut-offs and in vivo evaluation in murine pharmacology models for pharmacokinetic and dynamic profiling.

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REFERENCES

1. Trouiller P, Olliaro P, Torrelee 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. Crop 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. Crop 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. Kos P, Vliegentack AJ, Berghoe DV, Maes L. Anti-infective potential of medicinal plants used in Terai forest of western Nepal. J Ethnobiol Ethnomed 2010;6:268-75.
10. Patwardhan B, Mashekar RA. Traditional medicine-inspired approaches to drug discovery: Can Ayurveda show the way forward? Drug Discov Today 2003;8:804-11.
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, Triawani 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 Anthology 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, Iseosimi OI, Osugwu 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.
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. J 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;1:1-5.
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 jatropane diterpenoids from Pedilanthus tithymaloides. J Nat Prod 2007;70:1434-8.
48. Adzu B, Zakariya S, Aita I, Katsayal U. Assessing the potency of Pedilanthus tithymaloides latex against Plasmodium berghei infected mice. Int J Biol and Chem Sci 2008;2:216-9.
49. Sauvain M, Moretti C, Mizion V, Ruiz E, Balanza E. Jatropha grossidentata and jatrophone from Jatropha isabellii. Phytother Res 1996;10:378.
50. Pettit GR, Ducki S, Tan R, Gerdella 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.