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**Original Article**

# Antiviral Activity of Some Plants Used in Nepalese Traditional Medicine

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Methanolic extracts of 41 plant species belonging to 27 families used in the traditional medicine in Nepal have been investigated for *in vitro* antiviral activity against Herpes simplex virus type 1 (HSV-1) and influenza virus A by dye uptake assay in the systems HSV-1/Vero cells and influenza virus A/MDCK cells. The extracts of *Astilbe rivularis, Bergenia ciliata, Cassiope fastigiata* and *Thymus linearis* showed potent anti-herpes viral activity. The extracts of *Allium oreoprasum, Androsace strigilosa, Asparagus filicinus, Astilbe rivularis, Bergenia ciliata* and *Verbascum thapsus* exhibited strong anti-influenza viral activity. Only the extracts of *A. rivularis* and *B. ciliata* demonstrated remarkable activity against both viruses.

**Keywords:** anti-herpes – anti-influenza – anti-viral – medicinal plant

## Introduction

Plants have long been used as a source of medicine from ancient time to today all over the world. In developing countries the availability of modern medicines is limited. So traditional medicine is still the mainstay of health care and most drugs come from plants. Although many plants have long been recognized and widely used in Nepalese traditional medicine, some are relatively unexplored and not arrived to mainstream medicine (1). Therefore, the search on new drugs must be continued and natural products from plants, microorganisms, fungi and animals can be the source of innovative and powerful therapeutic agents for newer, safer and affordable medicines (2,3). On the other hand the screening of plants as a possible source of antiviral drugs has led to the discovery of potent inhibitors of *in vitro* viral growth (4–11).

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Therefore, the present investigation was carried out to assess the antiviral effects of some native plants used by the local people belonging to Gurungs and Thakalis of Manang and Mustang districts that lie in the Annapurna Conservation Area Project (ACAP). Permission for the field study as well as the collection of voucher specimens was received from the headquarters of ACAP in Pokhara. The plants were selected on the basis of ethnopharmacological records, so the prospect of finding new bioactive compounds is always promising.

## Methods

### Plant Materials and Preparation of Extracts

The plants were collected in the Manang and Mustang district of Nepal during summer 2004 and 2005 and dried in shady place. The plants were authenticated by Prof. Ram P. Chaudhary, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal and voucher specimens were deposited in the Tribhuvan University

Central Herbarium (TUCH), Kirtipur, Nepal. The name of the plants, respective families, the parts used for the extract preparation and traditional uses of the plants are listed in Table 1.

The dried and powdered plant material (each 10 g) was extracted successively with *n*-hexane, dichloromethane and methanol in a soxhlet extractor for each 8 h. Evaporation of the solvent followed by drying in vacuum gave the respective crude dry extract. Only methanol extract was used for the antiviral assay, *n*-hexane and dichloromethane extracts were not included because of their insolubility in medium and high toxicity to the cells. Each 2 mg of the extract was dissolved in 10 µl dimethylsulfoxide (DMSO) before adding tissue culture medium supplemented with 2% fetal calf serum (FCS, GIBCO Life science technologies, Paisley, UK) and stocked at a concentration of 2 mg ml$^{-1}$.

### Cells and Viruses

Madine–darby canine kidney (MDCK) and African green monkey kidney (Vero) cells (cell bank of the Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany) were maintained in Eagle's minimal essential medium (MEM) supplemented with 5% FCS (GIBCO, Paisley, UK). The exponentially growing cells were harvested and seeded at a cell density of 60 000/well in a 96 well microtiter plate (8 mm diameter, Falcon Plastic, NJ) and incubated for 24 h at 37°C with 5% carbondioxide in a 90% humidified chamber so as to form confluent monolayers.

Human influenza virus A/WSN/33 (H1N1) London was obtained from the strain collection of the Institute of Medical Microbiology, University Greifswald, Germany, and propagated in embryonated hen eggs for 72 h. The infected allantoic fluids were harvested, the hemagglutination (HA) titer and virus infectivity were determined on MDCK cells and the virus stock was stored at −70°C.

Herpes simplex virus type 1 (HSV-1, strain KOS) was obtained from the strain collection of the Consiliar and Reference Center for Alpha Herpes Virus Infection, Institute of Virology and Antiviral Therapy, University Jena, Germany and propagated in Vero cells. The virus infected cells were frozen and thawed and the virus suspension was titrated on Vero cells and stored at −70°C (7).

### Cytotoxicity Assay

The cellular toxicity of extracts on Vero and on MDCK cells was assessed by dye uptake method using neutral red (12) in 96-well tissue culture plates (8 mm diameter, Falcon Plastic, NJ). Only living cells are able to manage the active uptake of neutral red. Confluent monolayers of cells were treated with 100 µl 2-fold serial dilutions of extracts prepared at concentrations of 200, 100, 50 and 25 µg ml$^{-1}$ in four replicates and incubated at 37°C in a humidified atmosphere of 5% $CO_2$ for 72 h. The supernatant was removed and 200 µl neutral red solution (0.005%) in optimum was added. The microtiter plate was further incubated for 3 h at 37°C. After removal of the supernatant, the dye incorporated by the viable cells was extracted with 100 µl ethanol/water/glacial acetic acid solution (50 : 50 : 1) by shaking for 15 min. The absorbance was measured on an ELISA reader using Ascent software at 540 nm. The cytotoxic concentration that caused the reduction of viable cells by 50% [$CC_{50}$] was calculated from dose–response curve.

### Antiviral Assay

Antiviral activity was determined by dye uptake assay using neutral red as described by Mothana *et al.* (7). Non-cytotoxic extracts were tested in concentrations of 100, 50, 25, 12.5 and 6.25 µg ml$^{-1}$. The antiviral tests of cytotoxic extracts started with the half of the individual $CC_{50}$. The extracts were diluted 1 : 2 by medium. Confluent monolayers of Vero and MDCK cells were treated with 100 µl of extracts in four replicates for 30 min. After that Vero cells were infected with 30 $TCID_{50}$ of HSV-1 and MDCK cells with 30 $TCID_{50}$ of influenza virus A and incubated for 72 h at 37°C. $TCID_{50}$ (tissue culture infectious dose) is the virus dose that leads to the infection of 50% of the cells. The virus suspension and dilution medium without samples were added, respectively, to the cell cultures to serve as the virus control and cell control. The supernatant was replaced by 200 µl neutral red solution (0.005%) and the cells were incubated for 3 h at 37°C. After removal of the supernatant, the dye incorporated by viable cells was eluted with 100 µl ethanol/water/glacial acetic acid solution (50 : 50 : 1) by shaking for 15 min. The absorbance was measured at 540 nm and the percentage protection was calculated by the following formula (13):

$$(OD_T)_V - (OD_C)_V / (OD_C)_M - (OD_C)_V \times 100\ (\%).$$

where, $(OD_T)_V$, $(OD_C)_V$ and $(OD_C)_M$ correspond to absorbances in virus infected cells with test compounds, virus infected cells without test compounds and the mock infected control (assay without viruses), respectively.

Amantadine HCl and acyclovir were used as reference compounds in concentrations of 0.1, 1, 10 and 100 µg ml$^{-1}$.

## Results

### Cytotoxicity of Extracts for Vero Cells

In this study, 43 methanolic extracts from 41 different plant species belonging to 27 families (Table 1) were

**Table 1.** Name of the plants, respective families, parts used for extraction and major traditional use(s)

| Name of plant | Family | Collected part(s) | Vernacular (Gurung) name | Voucher no. | Major traditional use(s) |
|---|---|---|---|---|---|
| *Abies spectabilis* Spach. | Pinaceae | Leaves | Kye | 342 | Bone fracture |
| *Allium oreoprasum* Schrenk | Alliaceae | Whole plant | Lungho | 2104 | Cough, cold, sore throat |
| *Allium prattii* C.H. Wright | Alliaceae | Whole plant | Banlasun | 493 | Vegetables |
| *Anaphalis busua* DC. | Asteraceae | Leaves | Phosorosan | 463 | Cough, cold, sore throat |
| *Anaphalis busua* DC. | Asteraceae | Flowers | Phorosan | 463 | Cough, cold, sore throat |
| *Androsace strigilosa* Franch. | Primulaceae | Whole plant | Gadhikanakyo | 169 | Fever, edema |
| *Anemone rivularis* Buch.-Ham. ex DC. | Ranunculaceae | Roots | Angsoup | 492 | Cough, cold, stomachache |
| *Arisaema flavum* Schott | Araceae | Tubers | Timtry | 618 | Skin disease, wounds |
| *Artemisia caruifolia* Roxb. | Asteraceae | Whole plants | Bajha | 421 | Incense |
| *Asparagus filicinus* Buch.-Ham. ex D. Don | Asparagaceae | Tubers | Nirshing | 2125 | Tonic, menstrual problem |
| *Astilbe rivularis* Buch.-Ham. ex D. Don | Saxifragaceae | Rhizomes | Bhadhangoo | 2070 | Headache, improve fertility |
| *Bergenia ciliata* (Haw.) Sternb. | Saxifragaceae | Rhizomes | Pakhanved | 2075 | Diarrhea, dysentery, stomachache |
| *Bistorta affinis* Greene | Polygonaceae | Root | Khaldi | 203 | Cough, cold, tonsillitis, fever |
| *Cassiope fastigiata* D. Don | Ericaceae | Aerial parts | Sunpathi | 433 | Incense |
| *Clinopodium umbrosum* Matsum | Lamiaceae | Aerial parts | Sarshang | 155 | High blood pressure, pain, inflammation of body |
| *Cotoneaster integrifolius* (Roxb.) Klotz | Rosaceae | Fruits | Tsharsin | 168 | Edible |
| *Delphinium brunonianum* Royle | Ranunculaceae | Whole plant | Ponmar | 262 | Fever, jaundice |
| *Dicranostigma lactucoides* Hook.f. & Thomson | Papaveraceae | Whole plant | Rhafendhi | 105 | Easy delivery of baby (animals only) |
| *Euphorbia longifolia* D. Don | Euphorbiaceae | Root | Dhurbi | 2018 | Cough, cold, fever, skin disease |
| *Geranium donianum* Sweet | Geraniaceae | Aerial part | Kagheshurti | 153 | Gingivitis, toothache |
| *Hyoscyamus niger* var. *agrestis* (Kit.) Beck | Solanaceae | Flower | Lantang | 2236 | Anti-inflammatory |
| *Juniperus squamata* Buch.-Ham. ex Lamb | Cupressaceae | Aerial part | Sukri | 265 | Fever, cough, cold, skin disease |
| *Maharanga emodi* DC. | Boraginaceae | Roots | Maharangi (Nepali) | 2071 | Ear pain |
| *Morina longifolia* Wall. ex DC. | Morinaceae | Roots | Changtser goepa | | Edema, stomachache, headache |
| *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong | Scrophulariaceae | Roots | Kutki | 431 | Fever, cough, cold, tonsillitis |
| *Oxytropis williamsii* I. T. Vassilchenko | Fabaceae | Whole plants | Sinshi | 329 | Wound healing, coagulate blood |
| *Primula involucrata* Sw. ex Duby | Primulaceae | Whole plants | Chyonker | 178 | Vegetable |
| *Rhododendron anthopogon* D. Don | Ericaceae | Aerial part | Palu, Sangalin | 210 | Reduce blood pressure, fever, inflammation |
| *Rhododendron lepidotum* Wall. & G. Don | Ericaceae | Aerial part | Bhaiunakpo | 2122 | Fever, cough, cold, tonsillitis |
| *Rosa macrophylla* Lindl. | Rosaceae | Flower | Seghu | 343 | Fever, diarrhea, dysentery |
| *Rosa macrophylla* Lindl. | Rosaceae | Fruits | Seghu | 343 | Nutrition in cold, cough |
| *Rosa sericea* Lindl. | Rosaceae | Fruits | Sewa | 102 | Diarrhea, dysentery, stomachache, dyspepsia |
| *Rubus foliolosus* D. Don | Rosaceae | Root | Mapalan | 2019 | Fever, dyspepsis, cough, cold, vertigo |
| *Salix serpyllum* Andersson | Salicaceae | Aerial part | Langmanackpo | 2015 | Stomachache, diarrhea, dysentery |
| *Saussurea auriculata* (DC.) Sch. Bip. | Asteraceae | Whole plant | Ta | 283 | Blood circulation |
| *Saussurea fastuosa* (Decne) Sch. Bip | Asteraceae | Aerial part | Singamindro | 303 | Cut, bleeding |
| *Swertia ciliata* (G. Don) B. L. Burtt | Gentianaceae | Whole plant | Tiktha | 311 | Fever due to stomach and liver disorder |
| *Thalictrum cultratum* Wall. | Ranunculaceae | Roots and stem | Nagghunensa | 121 | Fever, diarrhea (for animal only) |
| *Thymus linearis* Benth. | Lamiaceae | Whole plant | Akhino | 126 | Eye infection |
| *Urtica dioica* L. | Urticaceae | Leaves | Polo | 409 | Cough, cold |
| *Valeriana jatamansi* Jones | Valerianaceae | Roots | Nappu | 2072 | Sedative, headache |
| *Verbascum thapsus* L. | Scrophulariaceae | Aerial part | Yugisingh | 195 | Wound healing, urinary disease, edema |
| *Zanthoxylum armatum* DC. | Rutaceae | Fruits | Prumo | 2183 | Cough, cold, tonsillitis |

screened for their antiviral activity against herpes simplex virus and influenza virus A by dye uptake assay. By methanolic extraction, a broad spectrum of compounds with different polarity can be obtained. As prerequisite for antiviral tests, the cytotoxicity of the extracts against virus-host cells was investigated. The results are summarized in Table 2.

The extracts of *Androsace strigilosa, Anemone rivularis, Delphinium brunonianum, Euphorbia longifolia* and *Thalictrum cultratum* exhibited strong cytotoxicity in Vero cells with $CC_{50}$ (the concentration that causes the reduction of viable cells by 50%) ranging from 12.5 to 25 $\mu g\,ml^{-1}$. A moderate cytotoxicity was observed for the extracts of *Asparagus filicinus, Bergenia ciliata, Primula involucrata* and *Saussurea auriculata* with $CC_{50}$ ranging from 30 to 50 $\mu g\,ml^{-1}$. Other eight extracts showed very mild toxicity while rest of the extracts were non-toxic at 100 $\mu g\,ml^{-1}$.

### Cytotoxicity of Extracts for MDCK Cells

Similarly, in MDCK cells extracts of *Artemisia caruifolia, D. brunonianum* and *E. longifolia* showed strong toxicity with $CC_{50}$ ranging from 19 to 25 $\mu g\,ml^{-1}$. A moderate toxicity was exhibited by the extracts of *A. strigilosa, A. rivularis, Asparagus filicinus, Dicranostigma lactucoides, Hyoscyamus niger, Thymus linearis* and *Zanthoxylum armatum* with $CC_{50}$ ranging from 30 to 50 $\mu g\,ml^{-1}$. Other three extracts demonstrated very low toxicity while rest of the extracts were non-toxic at 100 $\mu g\,ml^{-1}$.

### Antiviral Activity of Extracts Against HSV-1

Antiviral activity against HSV-1 was shown by 11 extracts at non-cytotoxic concentrations. The $IC_{50}$ values (the concentration that protects 50% of the cells against destruction by viruses) ranged from $<6.25$ to 82 $\mu g\,ml^{-1}$. The highest activity against HSV-1 with $IC_{50}$ values $<6.25\,\mu g\,ml^{-1}$ was observed for the extracts of *A. rivularis, B ciliata, Cassiope fastigiata* and *T. linearis*. Moderate activity was shown by *Cotoneaster integrifolius* ($IC_{50}$ 18 $\mu g\,ml^{-1}$) and *Clinopodium umbrosum* ($IC_{50}$ 19 $\mu g\,ml^{-1}$). Weak activity ($IC_{50}$ 50–82 $\mu g\,ml^{-1}$) was found in the extracts of *Bistorta affinis, Juniperus squamata, Oxytropis williamsii, Rhododendron anthopogon* and *Rubus foliolosus*.

### Antiviral Activity of Extracts Against Influenza Virus A

Antiviral activity against influenza virus A was shown by 20 extracts at non-cytotoxic concentrations. The $IC_{50}$ values ranged from $<6.25$ to 97 $\mu g\,ml^{-1}$. The highest activity was shown by the extracts of *A. filicinus, A. rivularis* and *Verbascum thapsus* with $IC_{50} < 6.25\,\mu g\,ml^{-1}$. In addition, the extracts of *Allium oreoprasum, A. strigilosa* and *B. ciliata* also exhibited high activity ($IC_{50}$ values from 8 to 10 $\mu g\,ml^{-1}$). Moderate activity ($IC_{50}$ values from 17 to 50 $\mu g\,ml^{-1}$) was demonstrated by 11 extracts. Weak activity ($IC_{50}$ values from 78 to 97 $\mu g\,ml^{-1}$) was shown by three extracts (Table 2).

The extracts of *A. rivularis* and *B. ciliata* were found to be highly active against both viruses.

## Discussion

The results of this work justify the potential of some of the investigated plants for the production of bioactive compounds. The phytochemical knowledge about these plants is so far very limited. The active principles present in *A. rivularis* are still unknown. Phytochemical investigation of *A. rivularis* revealed the presence of flavonoids, terpenoids and bergenin (14,15).

*Bergnia ciliata* is known to contain phenolic compounds (16). Polyphenols, especially high polymeric procyanidines possess strong anti-influenza viral activity (17), which is in agreement with our previous study (18). In our previous study (19), methanol–water extract of *Bergenia ligulata*, which is taxonomically closely related to *B. ciliata*, inhibited the growth of influenza virus A in cell culture with $IC_{50}$ of 10 $\mu g\,ml^{-1}$. The extract also inhibited the viral protein and nucleic acid synthesis (18). In the present study, the methanol extract of *B. ciliata* inhibited the influenza virus A and HSV-1 indicating that the genus *Bergenia* could be the source of potent antiviral drugs. Again potent activity of *A. rivularis* against both viruses indicated the high prospect of finding antiviral drugs in Saxifragaceae family.

No antiviral compounds have previously been isolated from *A. filicinus*. The plant is known to contain steroidal saponins (20,21), furostanol glycosides (22) and furostanosides (23,24). The phytochemicals possibly responsible for the high activity of *C. fastigiata* against HSV are not described. Some *Cassiope* species are reported to contain flavonoid glycosides (25). Similarly, the compounds responsible for the high anti-influenza viral activity of *A. oreoprasum* and *A. strigilosa* are not reported elsewhere.

Likewise, no antiviral constituents have been isolated from *C. integrifolius, C. umbrosum* and *T. linearis*. Other members of the genus *Cotoneaster*, have been found to possess phenolic glycosides (*Cotoneaster orbicularis*, 26), flavonols and isoflavones (*Cotoneaster simonsii*, 27). From the other member of the genus *Clinopodium, C. chinensis* var. *parviflorum*, oleanane triterpene saponins have been isolated (28).

Whereas for the extract of *V. thapsus*, antiherpes activity has been reported (29); our study revealed only the strong anti-influenza viral activity. However, no antiviral compounds have previously been isolated. The plant is known to contain phenylethanoid and lignan glycosides (30). On the other hand, the

**Table 2.** Antiviral activities of plants used in Nepalese ethnomedicine

| Plant extracts | Percentage yield of MeOH extract | Antiviral activity HSV-1/Vero cells | | Antiviral activity Influenza A/MDCK cells | |
|---|---|---|---|---|---|
| | | Cytotoxicity $CC_{50}$ (μg/ml)* | Antiviral activity $IC_{50}$ (μg/ml)† | Cytotoxicity $CC_{50}$ (μg/ml)* | Antiviral activity $IC_{50}$ (μg/ml)† |
| *Abies spectabilis* | 23.4 | > 100 | – | > 100 | 17 |
| *Allium oreoprasum* | 17.8 | > 100 | – | > 100 | 8 |
| *Allium prattii* | 7.5 | > 100 | – | > 100 | 97 |
| *Anaphalis busua* Leaves | 12.2 | > 100 | – | > 100 | – |
| *Anaphalis busua* Flower | 13.6 | > 100 | – | > 100 | – |
| *Androsace strigilosa* | 18.2 | 12.5 | – | 40 | 10 |
| *Anemone rivularis* | 14.5 | 21 | – | 40 | – |
| *Arisaema flavum* | 14.1 | > 100 | – | > 100 | – |
| *Artemisia caruifolia* | 12.3 | 92 | – | 22 | – |
| *Asparagus filicinus* | 18.7 | 40 | – | 30 | < 6.25 |
| *Astilbe rivularis* | 52.1 | 67 | < 6.25 | > 100 | < 6.25 |
| *Bergenia ciliata* | 33.2 | 35 | < 6.25 | > 100 | 9 |
| *Bistorta affinis* | 14.3 | > 100 | 80 | > 100 | 50 |
| *Cassiope fastigiata* | 18.2 | > 100 | < 6.25 | > 100 | 78 |
| *Clinopodium umbrosum* | 14.0 | 76 | 19 | > 100 | – |
| *Cotoneaster integrifolius* | 22.0 | > 100 | 18 | > 100 | 44 |
| *Delphinium brunonianum* | 12.3 | 11 | – | 25 | – |
| *Dicranostigma lactucoides* | 21.1 | 72 | – | 50 | – |
| *Euphorbia longifolia* | 18.5 | 25 | – | 19 | – |
| *Geranium donianum* | 24.4 | 89 | – | 69 | – |
| *Hyoscyamus niger* | 18.7 | > 100 | – | 50 | 40 |
| *Juniperus squamata* | 16.7 | > 100 | 82 | > 100 | – |
| *Maharanga emodi* | 14.7 | > 100 | – | > 100 | 29 |
| *Morina longifolia* | 5.9 | > 100 | – | > 100 | – |
| *Neopicrorhiza scrophulariiflora* | 38.3 | > 100 | – | > 100 | – |
| *Oxytropis williamsii* | 27.5 | > 100 | 78 | > 100 | 33 |
| *Primula involucrata* | 31.7 | 50 | – | 63 | – |
| *Rhododendron anthopogon* | 22.1 | > 100 | 50 | > 100 | 44 |
| *Rhododendron lepidotum* | 18.9 | 100 | – | > 100 | 58 |
| *Rosa macrophylla* Flower | 11.2 | 86 | – | > 100 | 45 |
| *Rosa macrophylla* Fruits | 10.5 | 74 | – | > 100 | – |
| *Rosa sericea* | 14.2 | > 100 | – | > 100 | – |
| *Rubus foliolosus* | 21.2 | > 100 | 50 | > 100 | – |
| *Salix serpyllum* | 26.2 | > 100 | – | > 100 | – |
| *Saussurea auriculata* | 11.4 | 31 | – | 100 | 42 |
| *Saussurea fastuosa* | 8.3 | > 100 | – | > 100 | – |
| *Swertia ciliata* | 6.2 | > 100 | – | > 100 | – |
| *Thalictrum cultratum* | 18.7 | 23 | – | 86 | 32 |
| *Thymus linearis* | 5.2 | 69 | 12.5 | 45 | – |
| *Urtica dioica* | 7.8 | > 100 | – | > 100 | – |
| *Valeriana jatamansi* | 50.1 | > 100 | – | > 100 | 20 |
| *Verbascum thapsus* | 12.3 | > 100 | – | > 100 | < 6.25 |
| *Zanthoxylum armatum* | 6.7 | > 100 | – | 36 | – |
| Acyclovir | | | 0.7 | | |
| Amantadine HCl | | | | | 16.8 |

*$CC_{50}$ = the concentration that causes the reduction of viable cells by 50%; †$IC_{50}$ = the concentration that protects 50% of the cells against destruction by viruses; – No measurable effect.
The values are the mean of four experiments.

phytochemicals responsible for anti-influenza viral activity could be different from anti-herpes activity and also the amount of active constituents present in the plants depends on the geographical distribution, season of collection and climatic and ecological condition at the collection site.

Looking at the chemical structures of the already identified compounds, most of these substances should be extracted by methanol. The foregoing extraction by more lipophilic solvents (*n*-hexane and dichlormethane) alleviates the methanolic extraction and the planned fractionation.

Comparing the use of plants in traditional medicine and their antiviral activity, a direct correlation could be established for some plants, e.g. *A. oreoprasum*, *A. strigilosa* (anti-influenza activity) and *T. linearis* (antiherpes activity). For other plants, e.g. *C. fastigiata*, which exhibited potent anti-herpes activity, this cannot be recognized till now.

The extracts that exhibited only medium and low activity, could also be the source of potential antiviral drugs because the bioactive compounds may be present in too low concentrations to show effective antiviral activity at non-toxic concentration. Further fractionation and separation of extract(s) may reveal potent antiviral activity (31).

Our results indicate that several plants used in Nepalese traditional medicine could be the lead to potential antiviral drugs, which possibly provide molecules with drug-like properties and with incredible structural diversity. Besides, the results are useful for rationalizing the use of medicinal plants in primary health care in Nepal. The phytochemical characterization of the extracts, the identification of the responsible bioactive compounds and the elucidation of the mode of action and quality standards are necessary.

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