A review on antiviral activity of the Himalayan medicinal plants traditionally used to treat bronchitis and related symptoms

Rahila Amber*, Muhammad Adnanb, Akash Tariqc,d and Sakina Mussaratb

*Department of Zoology, Kohat University of Science and Technology, bDepartment of Botany, Kohat University of Science and Technology, Kohat, Pakistan, cKey Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China and dUniversity of Chinese Academy of Sciences, Beijing, China

Keywords
antiviral activity; bronchitis; the Himalaya region; traditional knowledge

Correspondence
Muhammad Adnan, Department of Botany, Kohat University of Science and Technology, Kohat 26000, Pakistan.
E-mail: ghurzang@hotmail.com

Received April 27, 2016
Accepted October 23, 2016
doi: 10.1111/jphp.12669

Abstract

Objectives Bronchitis is a common respiratory tract infection of humans mainly caused by influenza virus, rhinovirus, adenovirus, coronavirus and respiratory syncytial virus. The aim of this review was to gather fragmented literature on ethnomedicinal plants used against bronchitis in the Himalayan region and their in-vitro validation against bronchitis causing viral pathogens.

Key findings Present review contains ethnomedicines of total 55 plants from different countries of the Himalayas. Most of the literature reported was from India followed by Pakistan, China and Nepal. Familiarly used plant families for bronchitis treatment in the Himalayan region were Leguminosae (six plants) and Lamiaceae (five plants). Leaves and roots were the most common parts used in ethnomedicines against bronchitis. Of these 55 plants, only six plants have been studied in vitro against viral pathogens causing bronchitis. Different compounds like monoterpenoids, flavonoids, triterpenoids, iridoid glycosides, sesquiterpenes, benzoic and phenolic compounds were reportedly isolated from these plant extracts having strong antiviral potential.

Summary The Himalayan regions possess variety of ethnomedicinal plants used against respiratory diseases, but still there are only few studies related with their in-vitro validation. We invite the attention of researchers for detailed ethnopharmacological and phytochemical studies on unexplored plants used to treat bronchitis for the development of novel antiviral drugs.

Introduction

Bronchitis is a respiratory disease of humans, in which mucous membrane of the bronchial tubes become inflamed that carry oxygen from trachea to lungs. The main aetiological agents for this disease are viruses including influenza, rhinoviruses, adenoviruses, coronavirus and respiratory syncytial virus while some bacteria including Mycoplasma pneumoniae, Chlamyphila pneumoniae and Bordetella pertussis account for about 10% of cases. Cigarette smoking, air pollution, occupational exposure to dust particles, irritants, fumes, polluted air particles and biomass fuels may also cause bronchitis. Bronchitis is slowly progressive and largely irreversible disease with clinical signs and symptoms like sore throat, runny nose, airway obstruction, tiredness, sensation of tightness, burning or dull pain in the chest under the breast bone, shortness of breath, fever, sputum production and sputum tenacity. According to World Health Organization report, respiratory tract infections are foremost cause of deaths among all contagious disease. Viral diseases are life-threatening diseases with high degree of complications to the humans because of its rapid outbreak throughout the developing world. Treatment of viral diseases is great challenge for the peoples even today because of different reasons like easily adaptations of virus, emergence of resistant viral pathogens, development of new viral strains, high cost and side effects of medicine, and host
resistance to antiviral drugs.\textsuperscript{10,11} Antibiotics are ineffective against viral infections because viral envelope and set of replicating machinery are completely different than that of bacteria.\textsuperscript{3} The common treatment for viral diseases includes antiviral drugs that do not destroy their target pathogen; instead, they inhibit their development, shorten infection and help prevent complications.\textsuperscript{12} Bacteria involved in causing bronchitis have also shown resistance against commonly used antibiotics (azithromycin, clarithromycin, tosufloxacin, minocycline, rifamycins and telithromycin) against respiratory tract infections.\textsuperscript{13,14} In recent years, emergence of resistant potential of viral and bacterial pathogens against commonly used drugs particularly for respiratory tract infections has increased, which is a major public health problem and great hurdle in the treatment of these diseases.\textsuperscript{12,15} Generally, resistance of viral pathogens to chemotherapy has been shown in immune-compromised individuals.\textsuperscript{16,17} The pathogenic resistance patterns and high cost of antiviral drugs encourage the use of medicinal plants all over the world.\textsuperscript{18}

In Asia, the medicinal plant species are abundantly found in the Himalayan forests that play a key role in the rural livelihood, by supplying diversity of valuable products for food and medicine.\textsuperscript{19} The Himalayan region is the largest mountain system blessed with large number of valuable medicinal plants because of its unique topographical, ecological, geographical, climatic and physiographical conditions.\textsuperscript{20} The Himalayan medicinal plants have very immense historical background, earliest evidences and records of using plants as medicines originated from the Himalayas and can found in old texts of ‘Rigveda’ written 6500 year ago.\textsuperscript{21} Local healers of the region have tremendous skills and knowledge of preparing traditional medicines for treating different kind of ailments. There is no exact figure about the number of the Himalayan medicinal plants being use for primary health care and livelihood, but most of the researchers documented that more than 10 000 Himalayan medicinal plants are supporting about 600 million people in the region.\textsuperscript{22}

Approximately 70–80\% people worldwide depend on medicinal plants to cure various human ailments including viral diseases.\textsuperscript{12} Herbal drugs have gained much importance due to their easily adaptability, low cost and fewer side reactions on patients.\textsuperscript{23} Various traditional medicine systems like Ayurveda and Chinese medicine have gained popularity and used in various parts of the world.\textsuperscript{24} Most of the people in the Himalayan region used medicinal plants traditionally to treat various diseases including bronchitis. Medicinal plants are considered as an important source of various bioactive compounds having antiviral properties, yet fewer studies have been done on antiviral potential of medicinal plants.\textsuperscript{15}

Literature is limited regarding the Himalayan medicinal plants used against viral diseases especially bronchitis. Main objectives of the present review were to gather utmost fragmented literature on the ethnomedicinal plants used to treat bronchitis in the Himalayan region and their in-vitro activity against viral pathogens and phytochemistry. This review will provide baseline information to the researchers about potential antiviral medicinal plants and will also disclose the scientific gaps in current knowledge that could lead towards the development of novel drugs for the welfare of human beings.

**Methodology**

Published data on traditionally used medicinal plants for bronchitis and their antiviral activity were collected from the online bibliographical databases: PubMed, Scopus, Google Scholar, Web of Science and floras of the Himalayan countries. Inside the databases, we used the keywords like medicinal plants, Himalaya, antiviral plant extracts and isolated compounds for bronchitis. Plant databases such as ‘Tropicos’ and ‘The Plant List’ (www.theplantlist.org) were searched for plant accepted names, synonyms and families.\textsuperscript{25} Total 87 articles were reviewed for this study, which were mostly published in English. We selected only those articles, in which complete information was given regarding the traditional use of medicinal plants in the Himalayan region and their antiviral activity. Two tables were developed using Microsoft Excel 2007 and Microsoft Word 2007. Table 1 contains information on the ethnomedicinal uses of selected plants used to treat bronchitis in the Himalayan region including information such as plant name, family, local name, habit, part used, study area and recipe formulation. Table 2 consisted of antiviral activity of plant extracts, concentration (µg/ml), inhibition (%) and active compounds tested against viral pathogens. Chemical structures of compounds were drawn using ChemDraw software (CambridgeSoft\textregistered ChemOffice2004/ChemDraw).

**Discussion**

**Ethnomedicinal plants used to treat bronchitis in the Himalayas**

The Himalayan region is considered to be the rich source of medicinal plants. Local peoples have strong traditional beliefs regarding high efficacy of ethnomedicinal plants used to treat bronchitis. Traditional healers believe that ethnomedicinal plants are more effective with fewer side effects as compared to modern allopathic drugs. Present review shows that 38 families are being used in the Himalayan belt across the different countries. Most of studies related to traditional uses have been carried out in India.
| Plant/Family/Local name | Study area | Habit/Part used | Recipe |
|-------------------------|------------|-----------------|---------|
| Abies pindrow (Royle ex D. Don) Royle/Pinaceae/Raisul[30] | Nepal, Garhwal Himalayas | Tree/Bark | Extract of bark is given orally |
| Acacia nilotica (L.) Delile/Leguminosae/Babool[59] | Bageshwar Valley (Kumaun Himalayas) of Uttarakhand, India | Tree/Bark | Bark’s extract is used |
| Achyranthes aspera L./Amaranthaceae/Lich Kura[30] | Garhwal Himalayas | Herb/Whole Plant | Plant decoction is given |
| Achyranthes bidentata Blume/Amaranthaceae/Kuru[59] | Bageshwar Valley (Kumaun Himalayas) of Uttarakhand, India | Herb/Root | Juice of root is used |
| Acorus calamus L./Acoraceae/Bojho, Katara, Bach[60] | Terai Forest in western Nepal | Herb/Root | Juice of root is given orally |
| Angelica glauca Edgew./Apiaceae/Chura[61] | Kashmir Himalaya | Herb/Root | Roots are used and combined with tonics for bronchitis |
| Artemisia indica Willd./Asteraceae/Tite Pat[60] | Terai Forest in western Nepal | Herb/Leaves | Leaf juice is given orally |
| Barleria cristata L./Acanthaceae/Porcupine Flower, Barleria[36] | Pauri Garhwal Himalayas | Shrub/Root | Root decoction is given |
| Betula utilis D. Don/Betulaceae/Bhojpatra, Bhooj, Himalayan Silver Birch[62] | Himachal Pradesh | Tree/Bark | Infusion of bark is given |
| Bidens pilosa L./Asteraceae/Blackjack, Spanish Needle[36] | Pauri Garhwal Himalayas | Herb/Whole Plant | Plant extract along with honey is given |
| Boerhavia diffusa L./Nyctaginaceae/Pundera[30] | Garhwal Himalayas | Herb/Whole Plant | Plant infusion is given |
| Bombax ceiba L./Malvaceae/Simal, Sema[36] | Terai Forest in western Nepal | Tree/Bark | Bark decoction is given orally |
| Cannabasis sativa L./Leguminosae/Bhang[38,59] | Vaidyas in Uhkimath Block, Uttarakhand | Herb/Leaves, Seeds | Leaves and seed extract with pepper, cumin seeds and cardamom is given |
| Cassia fistula L./Leguminosae/Amaltas, Raj Briksha[59] | Garhwal Himalayas | Tree/Fruit | Fruit pulp is given |
| Celastrus paniculatus Willd./Celastraceae/Sankhri[65] | Himachal Pradesh, India | Shrub/Seeds | Powder of seeds is given |
| Desmodium gangeticum (L.) DC./Leguminosae/Shalparni[59] | Bageshwar Valley (Kumaun Himalayas) of Uttarakhand, India | Herb/Root | Extract of root is given |
| Desmodium elegans DC./Leguminosae/Bhatul[59] | Bageshwar Valley (Kumaun Himalayas) of Uttarakhand, India | Herb/Whole Plant | Extract of plant is used |
| Ephedra gerardiana Wall. ex Stapf./Ephedraceae/Ephedra, Somlalat[39,64] | Pakistan, Himachal Pradesh | Shrub/Whole Plant | Powder of the crushed plant and some time its tea is used for relaxation of bronchial muscles |
| Euphorbia hirta L./Euphorbiaceae/Jatl-Doda[65] | Sewa River Catchment Area in the north-west Himalaya | Herb/Whole Plant | Decoction of plant is used |
| Euphorbia neriifolia L./Euphorbiaceae/Shreed[66] | Tarai Region of Kumaun, Uttarakhand, India | Shrub/Whole Plant | Cooked vegetable is given |
| Hedychium spicatum Sm./Zingiberaceae/Buru[67] | Garhwal Himalayas, Uttarakhand | Herb/Rhizome | Rhizome extract is taken orally |
| Hibiscus rosa-sinensis L./Malvaceae/Gurhal[59] | Bageshwar Valley (Kumaun Himalayas) of Uttarakhand, India | Shrub/Stem, Flower | Extract of stem and flower is used |
| Hippophae rhamnoides L./Elaeagnaceae/Buru[67] | Gilgit District of northern Pakistan | Shrub/Fruit | Syrup made from fruit mixed with Morus nigra L. fruit and sugar is used for bronchial congestion |
| Hyoscyamus niger L./Solanaceae/Henbane, Khorasani Ajwain[62] | Himachal Pradesh, India | Herb/Seeds | Seeds is principally employed as sedative in bronchitis |

© 2016 Royal Pharmaceutical Society, *Journal of Pharmacy and Pharmacology*, 69 (2017), pp. 109–122

Rahila Amber et al.

Antiviral activity of Himalayan medicinal plants
| Plant/Family/Local name | Study area | Habit/Part used | Recipe |
|-------------------------|------------|----------------|--------|
| Indigofera tinctoria L./Leguminosae/Neel[65] | Sewa River Catchment Area in the north-west Himalaya | Shrub/Whole Plant | Extract of plant is used |
| Jurinea ceratocarpa (Decne.) Benth. & Hook.f./Compositae/Turjit, Chholmung[68] | Nubra Valley – a cold arid zone of Himalaya | Shrub/Leaves | Leaf extract is given |
| Justicia adhatoda L./Acanthaceae/Asuro, Ross, Benkar, Brehankar, Baiker[60,70] | Terai Forest in western Nepal, Allai Valley, western Himalaya, Pakistan | Shrub/Whole Plant | Dried powder of entire plant parts is given. Roots and leaves are either given in decoction or powder form |
| Lantana camera L./Verbenaceae/Phoolwari[70] | Kumaun Himalayas of Uttarakhand | Shrub/Whole Plant | Decocition is given |
| Musa balbisiana Colla./Musaceae/Ban Kera[71] | Sikkim Himalayas of India | Herb/Leaves | Syrup is made from leaves and given |
| Myrica esculenta Buch.-Ham. ex D. Don/Myricaceae/Kafal, Kaphal[80] | Nepal Himalaya | Tree/Bark | Bark decoction is used |
| Myrtus communis Linn./Myrtaceae/Manoo[69] | Allai Valley, western Himalaya, Pakistan | Shrub/Whole Plant | Plant is taken internally in the treatment of bronchial congestion |
| Oberonia falconeri Hook.f./Orchidaceae/Hirni Chapli Amri[80] | Garhwal Himalayas | Herb/Whole Plant | Plant extract is given |
| Ocimum sanctum L. (Ocimum tenuiforum L.)/Lamiaceae/Tulsi[70] | Kumaun Himalayas of Uttarakhand | Herb/Leaves | Leave juice is used |
| Oroxylum indicum (L.) Kurz./Bignoniaceae/Sonpatha[66] | Western Himalaya, India | Herb/Leaves | Tea is made from leaves and used |
| Plantago major L./Plantaginaceae/Jabai[69] | Allai Valley, western Himalaya, Pakistan | Tree/Bark | Decocition of bark is given |
| Pinus roxburghii Sarg./Pinaceae/Chir Pine[86] | Pauri Garhwal Himalayas | Tree/Bark | Saw dust is given |
| Punica granatum L./Lythraceae/Anar[27,73] | Neelam Valley, Azad Jammu Kashmir, Kumaun Himalayas of Uttarakhand | Shrub/Whole Plant | Decocition of flower bud is given |
| Rheum nobile Hooker, f. & Thomson/Polygonaceae/Kenjo[74,75] | Himachal Pradesh India | Herb/Roots | The watery extract of roots is given orally |
| Rhododendron anthopogon D. Don/Ericaceae/Dhooopi[75] | Himachal Pradesh India | Shrub/Flowers, Leaves | Given orally |
| Tagetes minuta L./Asteraceae/Genda[65] | Sewa River Catchment Area in the north-west Himalaya | Herb/Flowers | Volatile oil of flower is used |
| Taxus wallichiana Zucc./Taxaceae/Himalayan Yew[76] | India, Nepalese Himalaya | Tree/Leaves | Leaves extract is given |
| Terminalia chebula Retz./Combretaceae/Harad[80] | Bageshwar Valley (Kumaun Himalayas) of Uttarakhand, India | Herb/Flower | Fruit powder is used |
| Thymus linearis Benth./Lamiaceae/Ban-Ajwain[77] | Himachal Pradesh, India | Herb/Whole Plant | Plant infusion or syrup is used |
| Toona ciliate M. Roem/Meliaceae/Toot[70] | Kumaun Himalayas of Uttarakhand | Tree/Bark | The extract of stem bark is given |
while rarely from Pakistan, China and Nepal due to less availability of published data (Table 1). Possible reasons for the high availability of ethnomedical data from India are: medicinal plants from India have been reported to be very useful in curing various human ailments including bronchitis, rich medicinal plants diversity as Himalaya region is mostly covered by India,\(^\text{26}\) most popular traditional systems of this country like Ayurveda, Siddha and Unani, strong traditional belief and dependency of local community on medicinal plants for curing diseases.\(^\text{26}\) The most widely used plant families for the treatment of bronchitis in the Himalayan region are Leguminosae (six plants), Lamiaceae (five plants) and Asteraceae (four plants). High number of medicinal plants in these families might be due to their higher abundance in the study area, presence of most active secondary metabolites like flavonoids in the Leguminosae;\(^\text{27}\) monoterpenoids, flavonoids and triterpenoids of Lamiaceae;\(^\text{28}\) and sesquiterpene lactones in the Asteraceae.\(^\text{29}\) Plants belonging to these families are not only used to treat bronchitis but also for many other human diseases in the Himalayan region.\(^\text{30–32}\) These families are not only preferred in the Himalayan region but in other countries as well for medicinal purposes due to antimicrobial activity of their plants.\(^\text{33,34}\) These families are not only used to treat bronchitis but also for many other human diseases in the Himalayan region.\(^\text{30–32}\) These families are not only preferred in the Himalayan region but in other countries as well for medicinal purposes due to antimicrobial activity of their plants.\(^\text{33,34}\) Medicinal plants used to treat bronchitis in the Himalayan region are mostly herbs (58%) as compared to shrubs (24%) and trees (18%) because they are easily collected, their higher abundance and efficacy against different human pathogens and popularity in traditional medicine system.\(^\text{35,36}\) Mostly whole plants are used traditionally in recipe formation to treat bronchitis, which is major threat to the conservation status of these medicinal plants. Almost all plant parts are found to have antiviral activity but most preferred plant parts used are leaves, roots, bark and flowers (Table 1). Different additives like water, sugar and honey are used in the recipe formation in order to reduce the bitter taste of plants as well as to reduce their toxic effects.\(^\text{37,38}\) Mixture of some plants is also used for recipes formulation in order to increase their efficacy due to synergistic effect of various compounds in these plants.\(^\text{199,40}\) As an example, leaves and seed extract of Cannabis sativa with pepper, cumin seeds and cardamom are given orally to treat bronchitis (Table 1). Powder, infusion, decoction and extract are common techniques for recipe formation in the Himalayan region. The most preferred techniques are decoction and extract in the study area that might be due to their easy administration or efficacy of dissolved bioactive compounds in extract.

### Antiviral activity of isolated compounds from plants

Data available on the Himalayan plants show that of 55 ethnomedically used plants, only six plants have been reportedly studied for their in-vitro activity against viral pathogens causing bronchitis. Whole plant and its different parts like leaves, flowers, aerial parts and rhizomes have been used for the preparation of extracts. Different solvents like aqueous, ethanol and methanol have been reportedly used for the extract preparation (Table 2). Methanol and ethanol are most preferable solvents for plant extraction due to their polar nature that ensures the release of wide variety of bioactive compounds from plants. Plants extracted with these solvents have been reported for their good antimicrobial activity.\(^\text{41,42}\) The plant extracts have been taken in different concentrations ranging from 6.25 \(\mu\)g/ml to 10 000 \(\mu\)g/ml, which showed strong inhibition ranges from 16.67% to 100% against adenovirus and influenza virus. Cos et al.\(^\text{42}\) studied the anti-infective potential of natural product and defined the criteria for good activity as 100 \(\mu\)g/ml for extracts and essential oils while 10 \(\mu\)g/ml for pure compounds and drugs. Among various techniques used for the detection of viral inhibition, dye uptake assay and cytopathic effect reduction assay

### Table 1 (Continued)

| Plant/Family/Local name | Study area | Habit/Part used | Recipe |
|-------------------------|------------|-----------------|--------|
| *Trifolium pratense* L./Leguminosae/ | Gilgit District of northern Areas | Herb/Flower | Powder of dried flowers is given |
| *Urtica dioica* L./Urticaceae/ | Ailai Valley, western Himalaya | Herb/Roots, Flowers | The juice of the roots or leaves mixed with honey or sugar is given |
| *Verbascum thapsus* L./Scrophulariaceae/ | Garhwal Himalayas | Herb/Whole Plant | Plant extract is used |
| *Viola canescens* Blume./Violaceae/ | Neelum Valley, Azad Jammu Kashmir | Herb/Leaves, Flowers | The decoction of leaves and flowers is given |
| *Viola pilosa* Blume./Violaceae/Kaura | Garhwal Himalayas | Herb/Whole Plant | Decoction of plant is given orally |
| *Zingiber officinale* Roscoe./ | Terai Forest of western Nepal | Herb/Rhizome | Rhizome is chewed in bronchial infections |

© 2016 Royal Pharmaceutical Society, Journal of Pharmacy and Pharmacology, 69 (2017), pp. 109–122
### Table 2 In-vitro screening of antiviral plants against viruses causing bronchitis

| Plant name          | Part used | Extract/ Compound/ Control drug | Chemical class | %age of composition of major comp. in the extracts | Concentration (µg/ml) | Inhibition (%) | Viral type                  | Techniques                  |
|---------------------|-----------|---------------------------------|----------------|---------------------------------------------------|----------------------|----------------|----------------------------|-----------------------------|
| **Hyoscyamus niger** | Flower    | Methanol                        |                | 18.7                                              | 40                   | 50             | Influenza A                | Dye uptake assay[78]         |
|                     |           | Acyclovir                       |                |                                                   |                      |                |                           |                             |
|                     |           | Amantadine HC                   |                |                                                   |                      |                |                           |                             |
| **Justicia adhatoda** | Leaves   | Methanol                        | Vasicine       | 0.026                                             | 10 000               | 100            | Influenza                  | Simultaneous assay[50]       |
|                     |           | Aqueous                         | Alkaloids      | 0.023                                             | 5000                 | 16.67          |                           |                             |
|                     |           |                                  |                |                                                   | 10 000               | 33             |                           |                             |
| **Ocimum basilicum** | Whole plant | Aqueous                         |                | 174.1                                             | 50                   |                | Adenovirus (ADV-3)        | Cytopathic effect reduction assay[28] |
|                     |           | Ethanol                         |                | >1000                                              |                      |                |                           |                             |
|                     |           | Linalool                        | Monoterpenoids | >95                                                | 24.4                 |                |                           |                             |
|                     |           | Apigenin                        | Flavonoids     | >95                                                | 11.1                 |                |                           |                             |
|                     |           | Ursolic acid                    | Triterpenoids  | >95                                                | >200                 |                |                           |                             |
|                     |           | 2',3'-Dideoxyxystidine          |                |                                                   |                      |                |                           |                             |
|                     |           | Aqueous                         |                | 26.7                                              | 50                   |                |                           |                             |
|                     |           | Ethanol                         |                | >200                                              |                      |                |                           |                             |
|                     |           | Linalool                        |                | >95                                                | 26.4                 |                |                           |                             |
|                     |           | Apigenin                        |                | >95                                                | 8.0                  |                |                           |                             |
|                     |           | Ursolic acid                    |                | >95                                                | 4.2                  |                |                           |                             |
|                     |           | 2',3'-Dideoxyxystidine          |                |                                                   |                      |                |                           |                             |
|                     |           | Aqueous                         |                | 129.6                                             | 50                   |                |                           |                             |
|                     |           | Ethanol                         |                | >200                                              |                      |                |                           |                             |
|                     |           | Linalool                        |                | >95                                                | 16.9                 |                |                           |                             |
|                     |           | Apigenin                        |                | >95                                                | 20.9                 |                |                           |                             |
|                     |           | Ursolic acid                    |                | >95                                                | 24.5                 |                |                           |                             |
|                     |           | Hot aqueous                     |                | 2500                                              | 32                   |                |                           |                             |
|                     |           | Methanol                        |                | 78                                                 | 28                   |                |                           |                             |
|                     |           | Hydro-methanol                  |                | 1250                                               | 30                   |                |                           |                             |
|                     |           | 2',3'-Dideoxyxystidine          |                | 28.2                                              | 50                   |                |                           |                             |
| **Plantago major**  | Whole plant | Aqueous                         | Iridoid glycoside | NA                                               | >1000                      | 50 | Adenovirus (ADV-3)  | XTT assay[10] |
|                     |           | DidoC                           | Phenolic       | 14.2                                              |                      |                |                           |                             |
|                     |           | Ferulic acid                    | Choralogic acid compounds | 76.0                          |                      |                |                           |                             |
|                     |           | p-coumaric acid                 |                |                                                   | >100                  |                |                           |                             |
|                     |           | Ursolic acid                    | Triterpenoids  | >40                                                |                      |                |                           |                             |
|                     |           | Vanillic acid                   | Benzoic compounds | >200                      |                      |                |                           |                             |
|                     |           | Aqueous                         | phenolic       | 5.3                                               |                      |                |                           |                             |
|                     |           | Aucubin                         | Phenolic       |                                                   | >1000                 | 50 | Adenovirus (ADV-8)  |                             |
|                     |           | Baicalin                         | Flavonoids     | >20                                               |                      |                |                           |                             |
|                     |           | Luteolin                        |                | >25                                               |                      |                |                           |                             |
|                     |           | Caffeic acid                    | Phenolic       |                                                   | 14.2                 |                |                           |                             |
|                     |           | Chlorogenic acid                |                |                                                   | 76.0                 |                |                           |                             |
|                     |           | Ulceric acid                    | Triterpenoids  | >40                                               |                      |                |                           |                             |
|                     |           | p-coumaric acid                 |                |                                                   | >200                 |                |                           |                             |
|                     |           | Ursolic acid                    | Benzoic compounds | >200                      |                      |                |                           |                             |
|                     |           | Aqueous                         | phenolic       |                                                   | >1000                 | 50 | Adenovirus (ADV-8)  |                             |
|                     |           | Aucubin                         | Phenolic       | >20                                               |                      |                |                           |                             |
|                     |           | Baicalin                         | Flavonoids     | >20                                               |                      |                |                           |                             |
|                     |           | Luteolin                        |                | >25                                               |                      |                |                           |                             |
| **Antiviral activity of Himalayan medicinal plants** Rahila Amber et al. | | | | | | | |
were the most common techniques (Table 2). These techniques are also used in other countries for in-vitro antiviral study.\[^{44,45}\] \(Zingiber~officinale\) has been investigated using plaque reduction test. In plaque reduction assay, crude fractions (pg per plate) were derived from the dose–response curve and from this the specific activity in units lg, where one unit of activity (1 u) was defined as the activity that reduced the number of plaques by 50% as compared to the virus controls. Flavan 5 was used as the positive control in the plaque reduction test.\[^{30}\]

Medicinal plants are the good source of various chemical compounds that provide basis for the development of new chemotherapeutic agents against various human pathogens.\[^{10,46}\] The mechanism of antiviral potential of plants extract or compounds varies among different viruses. Some phytochemical compounds of plants target viral envelope or membrane protein; others inhibit the formation of viral genome or stop attachment of virus to the host cellular machinery for reproduction while some destroy enzymes necessary for viral encoding.\[^{15,47}\]

Acute respiratory infections (ARI) remain serious problem and leading cause of death in young children throughout the world especially in developing countries. Most frequently reported viruses include rhinovirus, respiratory syncytial virus, influenza virus A and bocavirus. Anders \textit{et al.}\[^{48}\] demonstrated a high burden of ARI during the first year of life in southern Vietnam. Emerging drug-resistant potential of influenza virus led towards identification of novel antiviral agents. First-generation influenza antiviral agents such as amantadine and rimantadine act on viral M2 protein and stated as ion channel blockers. However, their side effects are associated with patient nervous system and gastrointestinal tract. Moreover, rapid emergence of viral resistance limited the benefits and usefulness of adamantanes in the prevention and treatment of influenza. Complementary and alternative medicines with ethnopharmacological background suggest novel platform for the development of antiviral drugs. Antiviral potential compounds present in medicinal plants work either alone or in synergistic manner. Effectiveness of several medicinal plants extracts is directly associated with synergistic properties of therapeutically active compounds and their derivatives. However, slight cytotoxicity observed in plant extracts investigated might be due to the presence of cytoprotective components. These phytocompounds and extracts could serve as potential source for the development of innovative

| Plant name          | Part used         | Extract/ Compound/ Control drug | Chemical class                      | %age of composition of major comp. in the extracts | Concentration (µg/ml) | Inhibition (%) | Viral type | Techniques         |
|---------------------|-------------------|---------------------------------|-------------------------------------|---------------------------------------------------|----------------------|----------------|------------|-------------------|
| Vanillic acid       | Aqueous           | ddC 10                          |                                     | >200                                              | 10                   | 50             | Adenovirus (ADV-11) |
| Aucubin             |                   |                                 |                                     | >1000                                             |                      | 50             |            |                   |
| Baicalein           |                   |                                 |                                     | >20                                               |                      |                |            |                   |
| Baicalin            |                   |                                 |                                     | >50                                               |                      |                |            |                   |
| Caffeic acid        |                   |                                 |                                     | >200                                              | 13.3                 |                |            |                   |
| Chlorogenic acid    |                   |                                 |                                     |                                                   |                      |                |            |                   |
| Ferulic acid        |                   |                                 |                                     |                                                   |                      |                |            |                   |
| Luteolin            |                   |                                 |                                     |                                                   |                      |                |            |                   |
| Oleanolic acid      |                   |                                 |                                     |                                                   |                      |                |            |                   |
| p-coumaric acid     |                   |                                 |                                     |                                                   |                      |                |            |                   |
| Ursolic acid        |                   |                                 |                                     |                                                   |                      |                |            |                   |
| Vanillic acid       |                   |                                 |                                     |                                                   |                      |                |            |                   |
| Verbascum thapsus   | Aerial parts      | Methanol Phenylethanoid and lignan glycosides | 6.25                                          | 50                                                |                      |                |            |                   |
| Zingiber officinale | Rhizome           | ar-curcumene Sesquiterpenes     | 20.4                                              | 0.90                                             |                      |                |            |                   |
|                     |                   | β-Sesquiphellandrene            |                                                   | 1.90                                             |                      |                |            |                   |
|                     |                   | α-Zingiberene                   |                                                   |                                                   | 14.3                 |                |            |                   |
|                     |                   | β-Bisabolene                    |                                                   |                                                   |                      |                |            |                   |
|                     |                   | Flavan Flavonoids               |                                                   |                                                   | 0.27                 |                |            |                   |
|                     |                   | 4, 6-Dichloroflavan             |                                                   |                                                   | 0.02                 |                |            |                   |
|                     |                   | Flavan 5                        |                                                   |                                                   | 0.02                 |                |            |                   |

© 2016 Royal Pharmaceutical Society, Journal of Pharmacy and Pharmacology, 69 (2017), pp. 109–122

Rahila Amber et al. Antiviral activity of Himalayan medicinal plants
antiviral drugs. Present review reported plant extracts likely to be promising candidates in the race of developing third-generation anti-influenza drugs, thus challenging the neuraminidase drug-resistant viruses in an effort to protect human health and the global economy. Present review showed that the Himalayan plants contain several bioactive substances like monoterpenoids, flavonoids, triterpenoids, iridoid glycosides, sesquiterpenes, benzoic and phenolic compounds having strong antiviral activity. Among traditionally used medicinal plants of the Himalayan region, phytochemicals of only four plants (*Justicia adhatoda*, *Ocimum basilicum*, *Plantago major* and *Zingiber officinale*) have been directly checked against viral pathogens (Table 2).

**Justicia adhatoda**

*Justicia adhatoda* is a well-known plant in Ayurvedic and Unani medicine, a shrub which is widespread throughout the tropical regions of south-east Asia. Leaves of this plant have been used extensively for the treatment of respiratory disorders. Simultaneous assay of leaves showed 33% reduction at a concentration of 10 000 µg/ml in aqueous extract and 16.67% reduction was observed from 1000 µg/ml to 5000 µg/ml, whereas 100% reduction was observed in methanolic extract at a concentration of 10 000 µg/ml. As the concentration decreased, inhibition also found to be decreased from 33.34% at 1 mg/ml to 16.67% at 5000 µg/ml. These data suggest that aqueous and methanolic extracts may directly interfere with protein envelope of viruses and not with sialic acid receptor at the cell surface. Only methanolic extract inhibited influenza virus infection by blocking viral attachment and inhibiting viral hemagglutinin (HA) protein. Vasicine alkaloids have been isolated from leaves, with percentage composition of 0.026% in methanol and 0.023% in aqueous extract which may be responsible for activity.

**Ocimum basilicum**

Sesquiterpenoid compounds like farnesol, caryophyllene, flavonoids such as apigenin (Figure 1), monoterpenes like linalool (Figure 2), cineole, fenchone, carvone, myrcene, geraniol and thujone, and triterpenoids such as ursolic acid (Figure 3) have been reportedly isolated from *Ocimum basilicum*. These compounds have been reported to inhibit various viral infections of DNA and RNA viruses. Only three types of secondary metabolites apigenin, linalool and ursolic acid have been checked directly against different types of human adenovirus involved in bronchitis. Among these ursolic acid showed maximum inhibition (50%) against adenovirus (ADV-8) at minimum concentration (4.2 µg/ml). Composition of all the isolated compounds is >95%. 2',3'-Dideoxycytidine is used as positive control and similar activity of ursolic acid (Table 2). Triterpenoid saponins of oleanane group inhibited the viral activity by inhibiting DNA synthesis, while urasane group inhibited viral protein capsid. Other compounds need further investigation against viral pathogens involved in bronchitis. Toxicological studies showed that higher doses of plant cause stomach irritation.

**Plantago major**

Phytochemicals isolated from *Plantago major* contain five classes of biologically active compounds: aucubin...
(Figure 4) belongs to class iridoid glycoside, baicalein, bai-
calin and luteolin are derivatives of flavons (Figure 5), and
vanillic acid (Figure 6) belongs to benzoic compounds and
derivatives of cinnamic acid (Figure 7) like caffeic acid,
chlorogenic acid, ferulic acid, p-coumaric acid, triterpenes
ursolic acid (Figure 3) and oleanolic acid (Figure 8). In-
vitro antiviral activity of these compounds has been
checked against three types of human adenovirus at differ-
ent concentrations. Although all compounds showed simi-
lar inhibition against adenovirus, but chlorogenic acid and
caffeic acid had shown 50% inhibition against adenovirus
at minimum concentration of about 13.3 and 14.2 µg/ml,
respectively (Table 2). The strong antiviral activity of phe-
nolic compounds might be correlated with the presence of
two hydroxyl groups on chlorogenic acid and caffeic acid
than ferulic acid and p-coumaric acid with only one hydro-
xyl group. Phenolic compounds found effective for their
antiviral activity not only against adenoviruses but also
against many other viral pathogens.[10,46] Many bioactive
chemical classes like flavonoids, triterpenes, sesquiterpenes,
phenolic and benzoic compounds isolated from many other
plants have been investigated for their antiviral activ-
ity.[10,15,28,47,51] Toxicological studies showed no toxicity of
methanolic extract with doses up 2 g/kg body weight in an
acute toxicity assay. Contrary to this result, another study
suggested that oral administration of an aqueous extract
(1000 mg/kg body weight) of leaves had shown highest
reduction in the total acidity of gastric fluid in rats.[54]
Another study reported that oral administration of aqueous
extract (2000 mg/kg) of Plantago major for total 40 days
did result in mortality of model organism.[52]

**Zingiber officinale Roscoe**

Three types of sesquiterpene derivatives (Figure 9) ar-cur-
cumene, β-sesquiphellandrene, α-zingiberene and β-bisa-
oblene and two types of flavonoids (Figure 10) flavan and
4, 6-dichloroflavan have been isolated from *Z. officinale* and investigated for their antiviral activity. All these compounds showed 50% inhibition against viral pathogens at different concentrations. 4, 6-Dichloroflavan was considered to be the most effective compound as it showed 50% inhibition against rhinovirus at very lowest concentration about 0.02 \( \mu \text{g/ml} \) (Table 2). The lipophilic nature of 4, 6-dichloroflavan has been attributed to its antirhinoviral activity as *Z. officinale* is known to contain various lipophilic secondary metabolites. Dichloroflavan isolated from other natural products has also been investigated against rhinovirus and showed its strongest ability to bind with most sensitive rhinovirus IB at their receptor sites. Many bioactive compounds isolated from higher plants have been checked for their antiviral activity particularly for their antirhinoviral activity. Available data showed that most of the antirhinoviral activity of plants attributed to the presence of flavonoids compounds. Administration of aqueous extracts either individually or in combination altered the relative weights of the heart, liver and kidney of the animals which is an indication of a toxic effect.

**Verbascum thapsus L**

Methanolic extract of *Verbascum thapsus* showed strong efficacy about 50% at minimum concentration of 6.25 \( \mu \text{g/ml} \) against *influenza A* virus. Phenylethanoid and lignan glycosides have been isolated from methanolic extract of *V. thapsus*. Phytoconstituents responsible for anti-influenza viral activity depend on the amount of active compounds present in the plants which alternatively depends on the geographical distribution, collected season, climatic and ecological condition at the site of plant collection. Further fractionation and separation of extract(s) may reveal potent antiviral activity from this plant. No toxicity has been observed for higher doses.

![Derivatives of cinnamic acid](image1)

![Oleanolic acid](image2)
Conclusions and Future Considerations

The Himalayan region contains variety of medicinal plants traditionally being used by the local people to treat bronchitis. However, literature is scanty regarding recipe formulation techniques, effective dosage and side effects of the Himalayan medicinal plants used against bronchitis. Therefore, it is necessary to carry out detailed ethnomedicinal studies covering all aspects which could be helpful for the patients and researchers. In addition, more focus should be given to widely distributed plants that might lead towards extraction of novel compounds due to geographical variation. Most of the ethnomedicinal studies were reported from India, and it is highly recommended that research activity in regard to antiviral medicinal plants should also be carried out in other countries of the Himalayan region. Very few plants have been screened in vitro and in vivo against viral pathogens involved in bronchitis. It is imperative to screen unexplored plants in detail not only for their in-vitro and in-vivo activity but also for their phytochemistry, toxicology and mechanism of actions.

Figure 9 Derivatives of sesquiterpenes.\textsuperscript{[30]}

Figure 10 Flavonoids.\textsuperscript{[55]}

© 2016 Royal Pharmaceutical Society, Journal of Pharmacy and Pharmacology, 69 (2017), pp. 109–122
Declarations

Conflicts of interest

The Authors have no conflicts to report.

Disclosure statement

The study had no ethical approval requirements.

References

1. Peiris JS et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003; 361: 1319–1325.
2. Alessandra P et al. Detection and typing by molecular techniques of respiratory viruses in children hospitalized for acute respiratory infection in Rome Italy. J Med Virol 2007; 79: 463–468.
3. Whitley RJ. HSV infections of women and their offspring: implications for a developed society. Proc Natl Acad Sci USA 1994; 91: 2441–2447.
4. Lanzetti M et al. Mate tea reduced acute lung inflammation in mice exposed to cigarette smoke. Nutrition 2008; 24: 375–381.
5. Yoshida T, Tudor RM. Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. Physiol Rev 2007; 87: 1047–1082.
6. Beaghehole R et al. The World Health Report 2004 – changing history. J Adv Nurs 2004; 48: 542–542.
7. Whitley RJ. HSV infections of women and their offspring: implications for a developed society. Proc Natl Acad Sci USA 1994; 91: 2441–2447.
8. Avila MM et al. Viral etiology in acute low respiratory infections in children from a closed community. Am Rev Respir Dis 1989; 140: 634–637.
9. Sathya B. A primitive approach on review of Siddha herbs, herbo-mineral formulation exhibiting antiviral activity. Int J Pharma Bio Sci 2014; 5: 138–147.
10. Chiang LC. Antiviral activity of Plantago major extracts and related compounds in vitro. Antiviral Res 2002; 55: 53–62.
11. Peera C, Effert T. Antiviral medicinal herbs and phytochemicals. J Pharmacogn 2012; 3: 1106–1118.
12. Wang X, Liu Z. Prevention and treatment of viral respiratory infections by traditional Chinese herbs. Chin Med J 2014; 127: 1344–1350.
13. Cao Bin et al. High prevalence of macrolide resistance in Mycoplasma pneumoniae isolates from adult and adolescent patients with respiratory tract infection in China. Clin Infect Dis 2010; 51: 189–194.
14. Yamaguchi H et al. Chlamydia pneumoniae resists antibiotics in lymphocytes. Antimicrob Agents Chemother 2003; 47: 1972–1975.
15. Ruwali P et al. Antiviral potential of medicinal plants, an overview. Int Res J Pharm 2013; 4: 8–16.
16. Zahradnik JM et al. Adenovirus infection in immunocompromised patients. Am J Med 1980; 68: 725–732.
17. Field HJ. Herpes simplex virus antiviral drug resistance-current trends and future prospects. J Clin Virol 2001; 21: 261–269.
18. Sumithra P et al. Antiviral and antioxident activities of two medicinal plants. Int J Carr Sci 2012; 256–261.
19. Kala CP. Ethnomedicinal botany of the Apatani in the eastern Himalayan region of India. J Ethnobiol Ethnomed 2005; 1: 11–18.
20. Kala CP, Mathur VB. Patterns of plant species distribution in the trans-Himalayan region of Ladakh, India. J Veg Sci 2002; 13: 751–754.
21. Malla SB, Shaky Pr. Medicinal plants. In: Majupuria TC, ed. Nepal Nature’s Paradise. Bangkok, Thailand: White Lotus Co. Ltd, 1984: 261–297.
22. Shengji P. Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. Pharm Biol 2001; 39: 74–79.
23. Edziri H et al. Antiviral activity of leaves extract of Marrubium alysson L. J Med Plants Res 2011; 5: 360–363.
24. Hudson JB. The use of herbal extract in use control of influenza. J Med Plants Res 2009; 3: 1189–1195.
25. www.Theplantlist.org
26. Gangwar RS, Joshi BD. Some medicinal flora in the riparian zone of river Ganga at Saptprishi Haridwar Uttarakhal. Himalayan J Environ Zool 2006; 20: 237–241.
27. Chew YL et al. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC Complement Altern Med 2011; 11: 1–10.
28. Chiang LC et al. Antiviral activities of extracts and selected pure constituents of Ocimum basilicum. Clin Exp Pharmacol Physiol 2005; 32: 811–816.
29. Fischer NH et al. The biogenesis and chemistry of sesquiterpene lactones. In: Herz W et al. eds Progress in the Chemistry of Organic Natural Products. Vienna: Springer-Verlag, 1979, 38: 47–320.
30. Radha B et al. Diversity and availability status of ethno-medicinal plants in the lohba range of Kedarnath Forest Division (KFD), Garhwal Himalaya. Glob J Res Med Plants Indig Med 2013; 2: 198–212.
31. Qureshi SJ et al. A survey of useful medicinal plants of Abbottabad in Northern Pakistan. J Sci 2008; 6: 39–51.
Antiviral activity of Himalayan medicinal plants

32. Marilena M et al. Impedance measurements to study the antimicrobial activity of essential oils from Lamioaceae and Compositae. Int J Food Microbiol 2001; 67: 187–195.
33. Stephanie I et al. Pharmacological activities and biologically active compounds of Bulgarian medicinal plants, phytochemistry. Adv Res 2006; 2: 87–103.
34. Rathore B et al. Indian herbal medicines; possible potent therapeutic agents for rheumatoid arthritis. J Clin Biochem Nutr 2007; 41: 12–17.
35. Ahmad H et al. Ethnobotanical study of upper Siran. J Herbs Spices Med Plants 2009; 15: 86–97.
36. Nazir A et al. Traditional uses of medicinal plants of Pauri Garhwal Uttarkhand. Nat Sci 2010; 8: 57–61.
37. Firdous A et al. Ethnopharmacological survey of Kajinaag range of Kashmir Himalaya, India. Int J Pharma Bio Sci 2012; 3: 442–449.
38. Sembwal DP et al. Medicinal plants used by local Vaidyas in Ukhimath block Uttarakhand. Indian J Tradit Knowl 2010; 9: 480–485.
39. Bisht VK et al. Traditional use of medicinal plants in district Chamoli Uttarakhand, India. J Med Plants Res 2013; 7: 918–929.
40. Lourens ACU et al. In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. J Ethnopharmacol 2004; 9: 253–258.
41. Parekh J et al. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J Biol 2005; 29: 203–210.
42. Richa S. Antiviral activity of crude extracts of Eugenia Jambolana Lam against highly pathogenic avian influenza (H5N1) virus. Indian J Exp Biol 2012; 50: 179–186.
43. Cos P et al. Anti-infective potential of natural products: how to develop a stronger in vitro ‘proof-of-concept’. J Ethnopharmacol 2006; 106: 290–302.
44. Waye MMY, Sing CW. Antiviral drugs for human Adenoviruses. Pharmaceuticals 2010; 3: 3343–3354.
45. Harbourne JB. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall, 1984.
46. Jassim SAA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol 2003; 95: 412–427.
47. Duke JA. Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. Boca Raton, FL: CRC Press, 1992.
48. Anders KL et al. Epidemiology and virology of acute respiratory infections during the first year of life: a birth cohort study in Vietnam. Pediatr Infect Dis J 2015; 34: 361–370.
49. Rajasekaran D et al. Identification of traditional medicinal plant extracts with novel anti-influenza activity. PLoS One 2013; 8: 79293.
50. Chavan R, Chowdhary A. In vitro inhibitory activity of Justicia adhatoda extracts against influenza virus infection and hemagglutination. Int J Pharm Sci Res 2014; 25: 231–236.
51. Lin YM. Antiviral activities of bilflavonoids. Planta Med 1999; 65: 120–125.
52. Garcia GM et al. Sub-chronic toxicity and test of eye irritability of leaf aqueous extract from Plantago major (Plantaginaceae). Rev Biol Trop 2003; 51: 635–638.
53. Escobar FM et al. Genotoxic evaluation of a methanolic extract of Verbascum thapsus using micronucleus test in mouse bone marrow. Nat Prod Commun 2011; 6: 989–991.
54. Koeasy MI et al. Gastoprotective effect of Plantago major L. against gastric injury induced by aspirin in rats. J Chem Acta 2013; 2: 86–91.
55. Denyer CV et al. Isolation of antihiviral sesquiterpenes from ginger (Zingiber Officinale). J Nat Prod 1994; 57: 658–662.
56. Bauer DJ et al. 4’, 6-Dichloroflavan (BW683C): a new anti-rhinovirus compound. Nature (London) 1981; 292: 369–370.
57. Plegsuriyakarn T et al. Cytotoxicity, toxicity, and anticancer activity of Zingiber officinale roscoe against cholangiocarcinoma. Asian Pac J Cancer Prev 2012; 13: 4597–4606.
58. Sulaiman FA et al. Antimicrobial and toxic potential of aqueous extracts of Allium sativum, Hibiscus sabdariffa and Zingiber officinale in wistar rats. J Taibah Univ Sci 2014; 8: 315–322.
59. Singh P, Attri BL. Survey on traditional uses of medicinal plants of Bageshwar valley (Kumaun Himalaya) of Uttarakhand, India. Int J Conserv Sci 2014; 5: 223–234.
60. Singh AG et al. An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal. J Ethnobiol Ethnomed 2012; 8: 19.
61. Bisht C, Badoni A. Medicinal strength of some alpine and sub-alpine zones of Western Himalaya. New York Sci J 2009; 2: 41–46.
62. Devi U, Thakur M. Exploration of ethno botanical uses of some wild plants from cold desert of Himachal Pradesh. Asian J Exp Biol Sci 2011; 2: 362–366.
63. Arti S et al. Studies on traditional knowledge of ethnomedicinal plants in Jawalamukhi Himachal Pradesh, India. Int Res J Biological Sci 2014; 3: 6–12.
64. Khan SM et al. Medicinal flora and ethnomedical knowledge in the Narang Valley, Western Himalaya, Pakistan. J Ethnobiol Ethnomed 2013; 9: 4.
65. Khan M et al. Medicinal plants of Sewa river catchment area in the Northwest Himalaya and its implication for conservation. Ethnobot Leafl 2009; 13: 13–39.
66. Mathur A, Joshi H. Ethnobotanical studies of the Tarai region of Kumaun Uttarakhand, India. Ethnobot Res Appl 2013; 11: 175–203.
67. Qureshi RA et al. Ethnobotanical studies of medicinal plants of Gilgit district and surrounding areas. Ethnobot Res Appl 2006; 5: 115–122.
68. Kumar PG et al. Ethnobotanical studies of Nubra Valley – a cold arid zone of Himalaya. Ethnobot Leaflets 2009; 13: 752–765.
69. Haq F. The ethno botanical uses of medicinal plants of Allai Valley, Western Himalaya Pakistan. Int J Plant Res 2012; 2: 21–34.
70. Singh HB et al. Folk medicinal plants in the Sikkim Himalayas of India. Asian Folklore Stud 2002; 61: 295–310.
71. Joshi M et al. Ethnomedicinal uses of plant resources of the Haigad
watershed in Kumaun Himalaya India. *Med Aromatic Plant Sci Biotechnol* 2010; 4: 43–46.

72. Husain SZ et al. Ethnobotanical properties and uses of medicinal plants of Morgah biodiversity park, Rawalpindi. *Pak J Bot* 2008; 40: 1897–1911.

73. Ahmad KS, Habib S. Indigenous knowledge of some medicinal plants of Himalaya Region, Dawarian Village Neelum Valley Azad Jammu and Kashmir, Pakistan. *Univers J Plant Sci* 2014; 2: 40–47.

74. Lepcha SR, Das AP. Ethno-medico-botanical exploration along the international borders to Tibet Autonomous Region of China and the kingdom of Bhutan with special reference to the Pangolakha Wildlife Sanctuary, East Sikkim. Recent Studies in Biodiversity and Traditional Knowledge in India Gour Mahavidyalaya, Malda 2011; 7: 257–270.

75. Kharwal AD, Rawat DS. Ethnobotanical studies and distribution of different *Rhododendron* species in Himachal Pradesh, India. *Plant Sci Feed* 2013; 3: 46–49.

76. Rahman S et al. *Taxus wallichiana* Zucc (Himalayan Yew): insights on its anti-microbial and pharmacological activities. *Altern Med* 2013; 1: 1–3.

77. Sharma PK, Lal B. Ethnobotanical on some medicinal and aromatic plants of Himachal Pradesh. *Indian J Tradit Knowl* 2005; 4: 424–428.

78. Rajbhandari M et al. Antiviral activity of some plants used in Nepalese traditional medicine. *Adv Access Publ* 2009; 6: 517–522.

79. Beate I. Structural determinants for activation and block of CFTR-mediated chloride currents by apigenin. *Am J Physiol Cell Physiol* 2000; 279: 838–846.

80. Chnag MY, Shen YL. Linalool exhibits cytotoxic effects by activating antitumor immunity. *Molecules* 2014; 19: 6694–6706.

81. Taralkar SV, Chattopadhyay S. A HPLC method for determination of ursolic acid and betulinic acids from their methanolic extracts of *Vitex negundo* Linn. *J Anal Bioanal Tech* 2012; 3: 1–6.

82. Johanna S. Extraction of iridoid glycosides and their determination by micellar electrokinetic capillary chromatography. *J Chromatogr* 2000; 868: 73–83.

83. Yasunori N. Effects of *Scutellariae radix* extract and its components (Bai-calein, Baicalin and Wogonin) on the experimental elevation of aqueous flare in pigmented rabbits. *Jpn J Ophthalmol* 2001; 45: 216–220.

84. Maria LAR et al. Degradation of vanillic acid and production of guaiaicol by microorganisms isolated from cork samples. *FEMS Microbiol Lett* 2003; 220: 49–55.

85. Jie L. Review article, Pharmacology of oleanolic acid and ursolic acid. *J Ethnopharmacol* 1995; 49: 57–68.