

Screening Phytochemical Constituents of 21 Medicinal Plants of *Trans*-Himalayan Region

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ABSTRACT

Alkaloids, tannins, flavonoids, saponins, steroids, and cardiac glycoside distribution in 1 high altitude medicinal plants belonging to different families (Apiaceae, Asteraceae, Crassulaceae, Lamiaceae, Rosaceae, Rubiaceae, Urticaceae, and Zygophyllaceae) were assessed and compared. The plants investigated were *Achillea millefolium*, *Artemesia dracunculus*, *Bidens pilosa*, *Carum carvi*, *Dracocephalum heterophyllum*, *Ferula jaeskiana*, *Gallium pauciflorum*, *Heracleum pinnatum*, *Hippophae rhamnoides*, *Inula racemosa*, *Mentha longifolia*, *Nepeta podostachys*, *Origanum vulgare*, *Peganum harmala*, *Rhodiola imbricata*, *Rhodiola heterodenta*, *Rosa webbiana*, *Rosa macrophylla*, *Rubia cordifolia*, *Tanacetum gracile*, and *Utrica hyperborea*, which have been widely used for time immemorial in the traditional Amchi system of medicine in the Ladakh region of India. Phytochemicals were qualitatively detected using aqueous extracts and solvent fractions of plants using various biochemical tests. These plants are a potential source of useful drugs. Future studies will isolate, identify, characterize and elucidate the structure of novel bioactive compounds. The significance of these plants in traditional medicine and the importance of the distribution of their chemical constituents are discussed in the context of the role of these plants in ethnomedicine in Ladakh.

Keywords: bioactive compounds, MAPs, traditional medicine, scientific investigation

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective or disease-preventive properties (phytochemicals). Among many biological hotspots around the world, the Himalayas and Western Ghats in India are regions of prime biodiversity concern. The Northern part of India harbors a great diversity of medicinal plants because of the majestic Himalayan range. The *trans*-Himalaya sustains about 337 species of medicinal plants (Kala 2002). This high proportion of medicinal plants among the existing flora, known for their medical purposes, is unique to India more than any other country in the world (Kala *et al.* 2006). Ladakh, the cold desert located in the Northernmost part of *trans*-Himalaya in Jammu and Kashmir State, is well known for its rich ethnobotanical wealth and the health care of the tribal population is mainly dependent on the traditional Amchi system of medicine. A great deal of traditional knowledge of the use of various plant species is still intact among the indigenous people; this is especially relevant in the mountainous areas such as the Himalayas due to poor accessibility of terrain and the comparatively slow rate of development (Farooque 2004). Therefore, much research is now devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them. *Achillea millefolium*, *Artemesia dracunculus*, *Bidens pilosa*, *Carum carvi*, *Dracocephalum heterophyllum*, *Ferula jaeskiana*, *Gallium pauciflorum*, *Heracleum pinnatum*, *Hippophae rhamnoides*, *Inula racemosa*, *Mentha longifolia*, *Nepeta podostachys*, *Origanum vulgare*, *Peganum harmala*, *Rhodiola imbricata*,

Rhodiola heterodenta, *Rosa webbiana*, *Rosa macrophylla*, *Rubia cordifolia*, *Tanacetum gracile*, and *Utrica hyperborea* are extensively used in the Amchi system of medicine in the Ladakh region of the Himalayas (Chaurasia *et al.* 2007). Their various uses in traditional medicine are reviewed in **Table 1**. The present study is a preliminary phytochemical screening of the important high altitude medicinal plants used in traditional medicine as an investigation to find a fundamental scientific basis for the use of these medicinal plants by defining the phytochemical constituents present in them.

MATERIALS AND METHODS

Collection of plant material

The leaves, stems and roots of each of these plants were collected from their natural habitat and from the herbal garden, DIHAR, Leh, Ladakh. All 21 samples were identified by the authors.

Extract preparation

Whole plants were shade dried, separated into leaves, stems and roots, then powdered using a pestle and mortar. The samples were extracted at room temperature with absolute ethanol, chloroform and distilled water (DW) for 24 hrs. Extracts prepared by different solvents were used to test for different compounds: DW (i.e., aqueous extract) for identification of tannins, flavonoids and saponins, chloroform extract for alkaloids and steroid tests, and ethanol extract for testing the presence of cardiac glycosides. The filtrates were obtained by using Whatman No. 1 filter paper.

Table 1 Review of the various medicinal uses of the high altitude medicinal plants.

Botanical name	Family	Vernacular name	Part used	Traditional medicinal usage
<i>Achillea millefolium</i>	Asteraceae	Yarrow (E), Chuang (L)	Leaves/flowers	Astringent, stimulant, used against heartburn, cold, colic, hysteria, epilepsy and rheumatism. Flowers as a tonic.
<i>Artemisia dracunculoides</i>	Asteraceae	Shersing or Burtse (L)	Leaves/roots	Roots as diuretic, for treatment of intestinal worms, lung diseases, mensural problems and toothache.
<i>Bidens pilosa</i>	Asteraceae	Local tea / Gurgur Chai	Leaves	As a local tea/Namkin chai
<i>Carum carvi</i>	Apiaceae	Konyot	Seeds	Fruits and seeds are used as febrifuge, eye vision and digestive.
<i>Dracocephalum heterophyllum</i>	Lamiaceae	Zinkzer (L)	Leaves/flowers	Cold, cough and headache.
<i>Ferula jaeskiana</i>	Apiaceae	Jangli Heeng / Chuklam (L)	Stem/roots	Roots used in rheumatism, Stem gum resin used to treat septic wounds and toothache.
<i>Galium pauciflorum</i>	Rubiaceae	Phomongo (L)	Stem/leaves	Used to cure intestinal parasites and reduces fever.
<i>Heracleum pinnatum</i>	Apiaceae	Spru-ma (L)	Roots	Used to treat inflammation and pain caused by vulnerable fever, checks haemorrhage and abdominal cramps.
<i>Hippophae rhamnoides</i>	Elagnaceae	Seabuckthorn (E) Tsermang (L)	Whole plant	Whole plant is medicinal. Fruits and seeds are used as blood purifier, against peptic ulcer, lung disorders, cuts and wounds. Anti inflammatory and improves digestion.
<i>Inula racemosa</i>	Asteraceae	Puskarmool (H) Manu (L)	Roots	Anti inflammatory, antiseptic, expectorant and diuretic.
<i>Mentha longifolia</i>	Lamiaceae	Jungli Pudhina (H)	Whole plant	Anti-dysenteric, carminative, antiseptic and stimulant. Used against fever and heat apoplexy.
<i>Nepeta podostachys</i>	Lamiaceae	Shangukaram	Aerial parts	Aromatic.
<i>Origanum vulgare</i>	Lamiaceae	Wild oregano / marjoram (E)	Leaves	Carminative and stimulates the flow of bile.
<i>Peganum harmala</i>	Zygophyllaceae	Wild rue (E) / Sepan (L)	Seeds	Used against fever, stomach complaints, anthelmintic, antiseptic, eye disorders, measles, asthma, rheumatism, joint pains, lactation and mensural problems. Diuretic and appetiser.
<i>Rhodiola imbricata</i>	Crassulaceae	Rose root or stone crop (E) / Shrolo (L)	Leaves/roots	Health tonic. Anti inflammatory, used for treatment of lung problems, cold, cough and restoring memory.
<i>Rhodiola heterodonta</i>	Crassulaceae	Rose root or stone crop (E) / Shrolo (L)	Leaves/roots	Treatment for lung problems, cold, fever and anti inflammatory.
<i>Rosa webbiana</i>	Rosaceae	Wild rose (E) / Siah (L)	Flower and fruits	Treatment of fever due to poison, food poisoning, inflammation of liver, hepatitis and jaundice.
<i>Rosa macrophylla</i>	Rosaceae	Wild rose (E) / Siah (L)	Flowers	Treatment of stomach pain
<i>Rubia cordifolia</i>	Rubiaceae	Manjith (H), Indian Maddar (E), Btsod (L)	Stems and roots	Effective against blood diseases, chest complaints, leucoderma, menstrual disorders, ulcers, stomach ache and urinary complaints.
<i>Tanacetum gracile</i>	Asteraceae	Khamchu (L)	Leaves/flowers	Used against intestinal worm.
<i>Urtica hyperborea</i>	Utricaceae	Stinging nettle (E), Dzatsutt or Zozot (L)	Leaves	Used against rheumatism and joint pain.

Source: Chaurasia *et al.* 2007; E = English; H = Hindi; L = Bodhi/Ladakhi

Phytochemical screening

Chemical tests were carried out on the aqueous and chloroform extracts and on the powdered specimens using standard procedures to identify the constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993). All the biochemical tests to test the qualitative presence of tannins, flavonoids, saponins, steroids, alkaloids and cardiac glycosides were carried out in triplicate; results were repeatable.

1. Test for tannins

0.5 g of the dried powdered samples was boiled in 20 ml of DW in a test tube and then filtered. A few drops of 0.1% FeCl₃ was added and observed for brownish-green or a blue-black colouration.

2. Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H₂SO₄. Yellow colouration observed in each extract indicated the presence of flavonoids. This colouration disappeared when the solution was left to stand.

3. Test for saponins

About 2 g of the powdered sample was boiled in 20 ml of DW in a water bath for 3 min and filtered. 10 ml of the filtrate was mixed with 5 ml of DW and shaken vigorously for 5 min to obtain a sta-

ble persistent froth, which was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion, which itself was a positive indicator for the presence of saponins.

4. Test for cardiac glycosides (Keller-Killani test)

5 ml of the ethanol extract was treated with 2 ml of glacial acetic acid containing one drop of FeCl₃ solution. This was underlaid with 1 ml of concentrated H₂SO₄ (98%). A brown ring of the interface indicates a deoxy sugar, characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer. The rings of violet or green indicate the presence of cardiac glycosides and the brown ring indicates the deoxy-ribo sugars.

5. Test for alkaloids (Wagner's test)

1 ml of the chloroform extract was mixed with 1 ml of Wagner's reagent. A positive reaction was indicated by a brown precipitate.

6. Test for steroids and terpenoids (Salkowski test)

5 ml of chloroform extract was mixed with 3 ml concentrated H₂SO₄ and shaken. A positive reaction was indicated by a red solution on standing.

Table 2 Qualitative analysis of phytochemicals of the high altitude medicinal plants (whole plants including flowers, leaves, stem and roots).

Plants	Alkaloids (chloroform extract)	Tannins (aqueous extract)	Flavonoids (aqueous extract)	Saponins (aqueous extract)	Cardiac glycoside (ethanol extract)	Steroids (chloroform extract)	Terpenoids (chloroform extract)
<i>Achillea millefolium</i>	+	+	+	+	+	+	+
<i>Artemisia dracunculus</i>	+	+	+	+	+	-	-
<i>Bidens pilosa</i>	+	+	+	-	+	-	-
<i>Carum carvi</i>	+	+	+	+	+	-	-
<i>Dracocephalum heterophyllum</i>	+	+	+	+	-	+	-
<i>Ferula jaeskiana</i>	+	+	+	+	+	+	+
<i>Gallium pauciflorum</i>	+	+	+	+	+	+	-
<i>Heracleum pinnatum</i>	+	+	+	+	-	+	-
<i>Hippophae rhamnoides</i>	+	+	+	-	-	-	+
<i>Inula racemosa</i>	+	+	-	+	-	+	-
<i>Mentha longifolia</i>	+	+	+	-	-	-	-
<i>Nepeta podostachys</i>	+	+	+	+	+	-	-
<i>Origanum vulgare</i>	+	+	+	+	-	+	-
<i>Peganum harmala</i>	+	-	+	+	+	-	-
<i>Rhodiola imbricata</i>	+	+	+	+	+	-	-
<i>Rhodiola heterodonta</i>	+	+	-	-	-	-	-
<i>Rosa webbiana</i>	+	+	+	+	+	-	-
<i>Rosa macrophylla</i>	+	+	+	+	+	-	-
<i>Rubia cordifolia</i>	+	+	+	+	-	-	-
<i>Tanacetum gracile</i>	+	+	+	+	+	+	+
<i>Utrica hyperborea</i>	+	+	-	+	+	+	+

+ Presence of constituent, - Absence of constituent

RESULTS AND DISCUSSION

The isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts at the earliest stages of separation and is thus economically very important.

The preliminary investigation conducted on the 21 high altitude medicinal plants revealed the presence of medicinally active phyto-constituents (**Table 2**). The results indicate that these plants were rich in alkaloids, tannins, flavonoids, saponins, steroids and cardiac glycosides, all known to exhibit medicinal as well as physiological activity. All the 21 plants screened gave a positive reaction for alkaloids. These are heterocyclic indole compounds which have proven pharmacological properties such as hypotensive, anti-convulsant, antiprotozoal, antimicrobial, and antimalarial activities (Lacqlercq *et al.* 1998; Frederich *et al.* 2002; Lata *et al.* 2010).

Acetogenins screened included tannins and flavonoids. Tannins are polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (Cowan 1999). Twenty one plants on investigation gave a positive reaction for tannins. Tannins possess general antimicrobial and antioxidant activities (Rievere *et al.* 2009). Low concentration of tannins can inhibit the growth of microorganisms, and act as an antifungal agent at higher concentrations by coagulating the microorganism's protoplasm (Adekunle and Ikumapayi 2006). Tannins may have potential value as cytotoxic and/or antineoplastic agents (Aguinaldo *et al.* 2005). Aside from the use of tannins as antimicrobial agents or in the prevention of dental caries, they are now being used in the manufacture of plastics, paints, ceramics and water softening agents (Bandarayanake 2002). The presence of tannins in the crude extracts examined may justify their therapeutic use to cure menstrual problems and toothache (*Artemisia dracunculus*, roots), toothache (*Ferula jaeskiana*, stem gum resin), blood diseases (*Rubia cordifolia*, roots) as well as use as an astringent (*Achillea millefolium*, leaves).

Seventeen samples tested positive for flavonoids which are known to possess anti-viral and anti-inflammatory properties. Flavonoids, a large group of naturally occurring

plant phenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones, possess numerous biological/pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, anti-allergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. Of these biological activities, the anti-inflammatory capacity of flavonoids has long been utilized in Chinese medicine and the cosmetic industry as a form of crude plant extracts (Kim *et al.* 2004; Aguinaldo *et al.* 2005; Moon *et al.* 2006; Veitch 2007; Jiang *et al.* 2008; Wu *et al.* 2008; Peteros and Mylene 2010). The presence of flavonoids in all crude plant extracts may confirm their folkloric use in treating rheumatism (*Ferula jaeskiana*, roots) and antiinflammatory (*Heracleum pinnatum*, roots; *Hippophae rhamnoides*, fruits) antioxidant (*Hippophae rhamnoides*, fruits and leaves).

Isoprenoids, including saponins and steroids, have expectorant and antidiabetic properties and are precursors for steroid hormones (Okwu 2001). 17 and 8 plant samples were positive for saponins and steroids, respectively (**Table 2**). The presence of saponins and steroids in the crude extracts examined may justify their therapeutic use as treatment of cold and cough as expectorant (*Dracocephalum heterophyllum*, flowers and leaves; *Inula racemosa*, roots) and treatment of asthma (*Peganum harmala*, seeds). 13 plant samples tested positive for cardiac glycosides, which are used in treating congestive heart failure and cardiac arrhythmia.

Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis, parasitic and protozoal infections and for their cytostatic effects. The disadvantage of using triterpenoids is the toxicity associated with their hemolytic and cytostatic properties (Dzubak *et al.* 2006). The presence of terpenoids in 4 crude plant extracts may confirm their traditional use in treatment against intestinal worm (*Tanacetum gracile*, leaves), stomach complaints (*Achillea millefolium*, leaves) and treatment of septic wounds (*Ferula jaeskianastem*, gum resin).

The medicinal plants studied here may be rich sources of phytochemicals, particularly alkaloids, tannins, flavonoids, steroids, cardiac glycosides and terpenoids which can be isolated and further screened for different kinds of biological activities, depending on their reported ethno-botanical and/or therapeutic uses and potential source of useful

drugs. Therefore, the data generated from these experiments provide a basic qualitative chemical basis for the wider use of these plants as therapeutic agents for treating various ailments. However, there is a need to carry out further advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds. Quantitative analyses of these phytochemicals may also be done as a guide as to which particular bioactive class of compounds may be subjected to subsequent target isolation. The antimicrobial activities of these plants for the treatment of diseases, as claimed by the traditional healers, are also being investigated.

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