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 jaekhiana, 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 (Farooquee 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 jaekhiana, 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. Extrakts 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.
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 Sofowora (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 solution 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 stable 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 underlayed with 5 ml of chloroform extract 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.

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.
Phytoconstituents of trans-Himalayan plants. Raj et al.

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 heterocyclic indole compounds which have proven pharmacological properties such as hypotensive, anti-inflammation, anti-convulsant, antitoxoal, antimicrobial, and antimalarial activities (Lacajloq 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 (Cowen 1999). Twenty one plants on investigation gave a positive reaction for tannins. Tannins possess general antimicrobial and antioxidant activities (Riever 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 cytototoxic and/or antineoplastic agents (Aguihaldo 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 (Bandarayakane 2002). The presence of tannins in the crude extracts examined may justify their therapeutic use to cure menstrual problems and toothache (Artemesia 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 anti-viral, anti-fungal, antioxidant, anti-inflammatory, anti-allergenic, antithrombic, antarcencogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. Of these biological activities, the anti-inflammatory capability 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; Petros and Mylene 2010). The presence of flavonoids in all crude plant extracts may confirm their folkloric use in treating rheumatism (Ferula jaeskiana, roots) and anti-inflammatory (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 cytotastic 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 intesinal 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

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

<table>
<thead>
<tr>
<th>Plants</th>
<th>Alkaloids (chloroform extract)</th>
<th>Tannins (aqueous extract)</th>
<th>Flavonoids (aqueous extract)</th>
<th>Saponins (aqueous extract)</th>
<th>Cardiac glycoside (ethanol extract)</th>
<th>Steroids (chloroform extract)</th>
<th>Triterpenoids (chloroform extract)</th>
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<td>Achillea millefolium</td>
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<td>Dracocephalum heterophyllum</td>
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<td>Ferula jaeskiana</td>
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<td>Peganum harmala</td>
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<td>Tanacetum gracile</td>
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* Presence of constituent, - Absence of constituent
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 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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