

Research Article

Secondary Metabolite Studies of Some Selected Plants of District Gilgit, Gilgit-Baltistan

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ABSTRACT

Five medicinal plants of belonging to families, *Cupressaceae*, *Asteraceae*, *Ranunculaceae*, *Polygonaceae* and *Saxifragaceae* were investigated for the presence of secondary metabolites namely alkaloids, amino acids, anthraquinone (free and as glycosides) ascorbic acid, carbohydrates, coumarins, flavonoids, phenolics, proteins, saponins and steroids in their aqueous, ethanolic and benzene extracts. *Cichorium intybus* L (Family *Asteraceae*), *Delphinium brunonianum* Royle (Family *Ranunculaceae*) and *Rheum speciforme* Royle (Family *Polygonaceae*) gave positive tests for alkaloids. Whereas *Capparis spinosa* L (Family *Cupressaceae*) and *Bergenia stracheyi* Hk (Family *Saxifragaceae*) showed negative tests for alkaloids. *Capparis spinosa* L fruits, gave positive results for carbohydrates and phenolics. *Cichorium intybus* L stem, had anthraquinone (free), carbohydrates, coumarins and phenolics. *Delphinium brunonianum* Royle, stem and leaves, showed positive results for amino acids, anthraquinone (as glycosides), carbohydrates, coumarins, phenolics, proteins and saponins. *Rheum speciforme* Royle, roots, had anthraquinone (free), ascorbic acid, carbohydrates, coumarins, flavonoids, phenolics, saponins and steroids. *Bergenia stracheyi* Hk, roots showed positive tests for amino acids, ascorbic acid, carbohydrates, phenolics and saponins.

INTRODUCTION

Men have always used natural resources of healing substances to cure human diseases. Efforts to cure the diseases by means of traditional phytotherapy have been made in all parts of the world (Heinrich, 2003; Abella *et al.*, 2000; Bodekar *et al.*, 2005). At present pharmacological knowledge of plant derived products of certain nations is used in the treatment of wide range of diseases (Shen-Ji, 2001; Uniyal *et al.*, 2006; Hameed *et al.*, 2011) including cancer, AIDS, Alzheimer's disease, alcoholism, etc. (Perry *et al.*, 1999; Baily *et al.*, 2005; Sojem and Gosai, 2006).

South Asia is home to many rich and traditional systems of medicines. Ayurvedic methods date back to 5000 B.C. long with the Unani, Siddha and Tibetan systems; they remain an important source of everyday health and livelihood for millions of people. Medicinal and aromatic plants, including trees, shrubs, grasses and vines, are a central resource for these traditional health systems, as well as for pharmaceutical (or allopathic) medicines. There are more than 6,000 plant species in South Asia with known medicinal uses (Rizvi *et al.*, 2007).

Plants are the source of energy for the animal kingdom. Plants can synthesize a number of chemical substances that have physiological importance (Kretovich 2005). Medicinal plants are well known to the local population and are used for the treatment of their ailments since time immemorial. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, are easily available and cheaper (Iwu *et al.*,

1999). Different plants have been used as a source of inspiration in the development of novel drugs (Robbers *et al.*, 1996). Many plant species have been evaluated for their antimicrobial activity during past twenty years (Castello *et al.*, 2002). Antimicrobial activity has been tested in some of the plants and showed positive results (Shahjehan and Ramesh, 2004), and (Ayandele and Adebisi, 2007).

The current study of secondary metabolites screening of plants well known for healing properties from District Gilgit was carried out for the first time on these plants. The purpose of the studies was to identify the plants that are rich in flavonoids, coumarins, ascorbic acid, polyphenols and saponins and could be used for anticancer, anti HIV, anti Alzheimer's disease and anti atherosclerosis purposes.

MATERIALS AND METHODS

Plant collection, identification and Extraction: The plant materials were collected from Naltar area of district Gilgit in the month of June and July, 2008 and identified in the Department of Biological Sciences, Karakoram International University. The samples were dried in shade and powdered by grinding with the help of Mortar and pestle. The powdered material was then used for the phytochemical screening.

Phytochemical Screening: Two grams of the powdered plant materials were extracted with 25 ml of methanol, ethanol and benzene separately, and extracts were concentrated over water bath. These extracts were used to

Table 1: Qualitative chemical tests for the presence/absence of Secondary Metabolites

Phytochemicals of Plant Analyzed	Alkaloids	Alkaloid	Amino acid	Anthraquinone free	Anthraquinone Glycoside	Ascorbic acid	Carbohydrates	Carbohydrate	Coumarins	Flavonoids	Phenolics	Phenolics	Protein	Saponins	Steroids
Experiment	Meyer's Reagent Test	Hager's Reagent Test	Ninhydrin Test	Borntrager Test	Modified Borntrager Test	Bic Acid Test	Molisch's Test	Fehling's Test	Coumarin Test	Flavonoid Test	Ferric Chloride Test	Lead Acetate Test	Million's Test	Saponin Test	Steroid Test
Observation	white or yellow precipitate	yellowish precipitate	pink color	Pink color in ammoniacal layer	pink color in ammoniacal layer	Deep violet color	Reddish violet ring	Brick red precipitate	deep green color	pink color	Green color	White precipitate	White precipitates	Soap like froth	Salkowski test Yellowish ring turned red
1 <i>Capparis spinosa</i> Fruit	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-
2 <i>Cichorium intybus</i> Stem	+	+	-	+	-	-	+	+	+	-	+	+	-	-	-
3 <i>Delphinium brunonianum</i> Stem and leaves	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-
4 <i>Rheum speciforme</i> Roots	+	+	-	+	-	+	+	+	+	-	+	+	-	+	+
5 <i>Bergenia stracheyi</i> Roots	-	-	+	-	-	+	+	+	-	-	+	+	-	+	+

Legend:

a: positive (+) means Present ; Negative (-) means absent

Maximum phytochemicals were found in roots of *Rheum speciforme*

detect the presence of alkaloids, amino acids, anthraquinone (free and as glycosides), ascorbic acid, carbohydrates, coumarins, flavonoids, phenolics, proteins, saponins and steroids (i.e. primary and secondary metabolites) from plant materials. The methods used were described by (Evans, 1996, and Parekh and Chanda, 2007).

The presence and absence of secondary metabolite constituents of powdered plant material was analyzed using the following standard methods

Carbohydrates: Leaves (500 mg) of sample were boiled in 30 ml DDW and filtered, 1 ml filtrate + 1 ml of Molisch's reagent + 1 ml conc. H₂SO₄. The presence of carbohydrate inferred by a reddish ring (Evans, 1996)

Reducing sugars: One milliliter of the above filtrate + 2 ml of Fehling's solution were boiled for 5 min. A brick red precipitate indicates the presence of reducing sugar (Evans, 1996).

Tanins: Two milliliters of the filtrate + 1 ml FeCl₃. A blue-black or greenish-black precipitate confirms tannins (Evans, 1996).

Saponins: Frothing test: 0.5 ml filtrate + 5 ml DDW, shaken for 30 s, persistent frothing indicates saponins (Evans, 1996).

Steroids: Liebermann–Burchard's test: 200 mg of the plant material in 10 ml chloroform, filtered. 2 ml filtrate + 2 ml acetic anhydride + 1 ml conc. H₂SO₄ are added to this. A blue-green ring shows the presence of steroids (Parekh and Chanda, 2007).

Alkaloids: Plant material (200 mg) was boiled in 20 ml of 1% H₂SO₄ in 50% ethanol and filtered; filtrate+ drops conc. NH₄OH + 20 ml chloroform were added and the two layers were separated. The chloroform layer was extracted with 20 ml dilute H₂SO₄. On addition of extract + 5 drops of Mayer's reagent, a creamy/brownish red/orange-red precipitate indicates the presence of

red/orange-red precipitate indicates the presence of alkaloids (Evans, 1996).

Anthraquinones: Borntrager's test: 100 mg of powdered plant in 5 ml of chloroform, filtered. 2 ml filtrate + 2 ml 10% NH₄ OH were added to this. A bright pink color confirms the presence of anthraquinones (Evans, 1996).

Glycosides: Keller–Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + 1 ml FeCl₃ + 1 ml conc. H₂ SO₄. A green-blue color indicates the presence of glycosides (Evans, 1996).

RESULTS AND DISCUSSIONS

The results of secondary metabolite screenings of five plants have been shown in Table 1. The secondary metabolite screening of methanol, ethanol, aqueous and benzene extracts showed the presence of maximum number of phytochemicals in *Delphinium brunonianum* Royle (Family *Ranunculaceae*). It showed positive tests for alkaloids, amino acids, anthraquinone glycoside, carbohydrates, coumarins, flavonoids, phenolics, saponins, and proteins.

Chemicals boost the host's anti-inflammatory defence and sensitize malignant cells to cytotoxic agents (Sexena et al., 2007). Plants (e.g. *Oxalis scorpioides*) containing alkaloids, tannins, glycosides, saponins and flavonoids have proven antibacterial activity against both gram positive and gram negative bacteria and fungi.

Capparis spinosa L. (family *Capparidaceae*) contained carbohydrate and phenolics. The plant was best known for edible bud and fruit (caper berry) which are usually consumed pickled. Caper (*Capparis spinosa* L.) and sea fennel (*Crithmum maritimum* L.) are part of the Mediterranean diet, which is known to be one of the healthiest diet in the world due to its relation with cardiovascular and arteriosclerosis diseases. By inhibiting the accumulation of harmful lipid oxidation products and increasing the level of bioavailable vitamin E, caper may have beneficial health effects, especially for people whose meals are rich in fats and red meats (Tesoriere et al 2007). Caper is used in phytomedicine as antioxidative, antifungal, antihepatotoxic, anti-inflammatory and antidiabetic (Eddouks et al. 2005). El-Ghorab et al. (2007) studied the antioxidant activity of essential oils of caper (*Capparis ovata* Desf. var. *canescens*) obtained from buds and leaves and reported its inhibitory activity on oxidation of hexanal and 1,1-diphenyl-2-picrylhydrazyl hydrate.

In Greek popular medicine an herbal tea made of caper root and young shoots is considered to be beneficial against rheumatism. Sprouts, roots, leaves and seeds are also used in the treatment of strangury and inflammation. Rutin is a powerful antioxidant bioflavonoid, believed to search out super-oxide radicals in the body. Capers contain more quercetin per weight than any other plants (USDA Database). Caper is used as analgesic, anthelmintic, antihaemorrhoidal, aperient, deobstruent, depurative, diuretic, emmenagogue, expectorant, tonic, vasoconstrictor. Rutin and quercetin may contribute to cancer prevention (Perry et al, 1999). Selenium present in capers at high concentration in comparison to other

vegetable products has also been associated with prevention of some forms of cancer. *Rheum speciforme* Royle (Family Polygonaceae) known as rhubarb, is used to stimulate blood circulation, treat jaundice and liver diseases. Its roots have purgative action and powdered roots are used for the treatment of constipation. In Chinese medicine the plant is considered potent for treating constipation, treat infections induced by microorganisms, reduce blood pressure and bad cholesterol in blood. *Bergenia stracheyi* Hk (Family *Saxifragaceae*) has potential to act as broad spectrum antimicrobial agent because of the presence of phytochemicals showed positive result for free anthraquinone, ascorbic acid, carbohydrates, phenolics, saponins and steroids. Rajkumar et al., (2010) extracted and evaluated the antioxidant activities from *Bergenia ciliata* rhizome. *Bergenia himalacia* has also been used for food due the presence of many kinds of amino acids and mineral elements which are helpful in health care (Yang et al., 2009). Bergenin is the most important pharmaceutical component, can be used to treat many diseases. Currently, it is already in clinical use, such as relieving a cough, eliminating phlegm, diminish inflammation, etc. (Jiang et al., 2010; Nazir et al., 2011; Yuan, 2011). *Delphinium brunonianum* Royle is locally used for cure of baldness, diarrhoea stomach ache and fever Hassan et al. Plants containing sterols and flavonoids present in fruits and vegetables reduce the risk of atherosclerosis, which is the build-up of fatty deposits in the artery walls (Goldberg et al., 2006). Food rich in polyphenols protect the humans against Alzheimer's disease. Presence of polyphenols in the plants under report could positively be used against this disease.

Phenols and flavonoids in olive act as antioxidant, anticancer, antimicrobial, and antibacterial agents (Valery, (2005) Vlassios et al., (2009)

Flavonoids are powerful antioxidants and their activity is related to their chemical structures. Dietary consumption of flavonoid rich nutrients as well as pure flavonoids was shown to attenuate the progression of atherosclerosis in animals (Huxley and Neil, 2003). *Chicorium intybus* root extracts has shown hepatoprotective effect against orotic acid –induced fatty liver in rats (Cha, et. al., 2003).

CONCLUSION

Some of the plants analyzed, have shown the presence of flavonoids, coumarins, ascorbic acid and saponins in addition to other secondary metabolites which are effective in the prevention of cancer, have anti-HIV, anti-tumor antihypertensive and anti-inflammatory properties. Saponins reduce the blood cholesterol level and risk of cancer as well.

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