Phytochemical Screening of *Ajuga bracteosa* Wall ex. Benth: An Endemic Medicinal Plant of Kashmir Himalaya

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Abstract

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. Many pharmaceutical agents have been discovered by screening natural products from plants. These natural products remain an important source of new drug leads and new chemical entities. Phytoconstituents obtained from plants have two categories i.e., primary and secondary. Primary constituents include chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial, antioxidant, antimutagenic and anti-inflammatory activities because of the presence of these phytoconstituents. Therefore, the present study was taken to have a preliminary investigation of the various phytoconstituents from the crude extracts of *Ajuga bracteosa* known as Jani- Adam. The crude extract showed the presence of various phytoconstituents like alkaloids, phenolics, tannin, cardiac glycosides, terpenes, flavonoids, saponin, steroids, carbohydrates and proteins. It is expected that the important phytochemical constituent recognized in *Ajuga bracteosa* found in Kashmir Himalaya will be very useful in the curing of various diseases of this region.

Keywords: Phytoconstituents, *Ajuga bracteosa*, antimutagenicity, EMS, micronucleus

Introduction

Traditional herbal medicine practitioners have described the therapeutic efficacy of many indigenous plants (Bharat and Parabia 2010). The plants are the source of synthetic and traditional herbal medicine and hence are useful for healing and curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.* 2000; Rao and Savithramma 2012; Choudhary *et al.* 2013). These phytochemicals are naturally present in all parts of medicinal plants viz. leaves, vegetables and roots. Phytochemicals are primary and secondary metabolites which are synthesised by the plants itself. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds include terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.* 2007). Terpenoids and phenols exhibit various important pharmacological activities like anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen 1997). Terpenoids are very important in attracting useful mites and consume the herbivorous insects (Kappers *et al.* 2005). Alkaloids are used as anesthetic agents and are found in medicinal plants (Herourat *et al.*, 1988; Kumbhar and Godghate, 2015).

Ajuga bracteosa Wall ex. Benth. of family Lamiaceae is commonly known as 'Bungle' in English and 'Jan-i-adam' in Kashmiri. It is a perennial erect, ascending hairy herb, often prostrate with oblanceolate or sub-spathulate leaves and grows up to 5-50 cm tall. It is distributed in subtropical and temperate regions Bhutan, Pakistan, Afghanistan, China, Malaysia at an altitude of 1300 m asl. In India, it abounds in western Himalaya, plains of

Punjab, upper Gangetic plains of India (Khare, 2007) and in Kashmir at an altitude of 1300 m (Chandel and Bagai 1999). It is found along roadsides, open slopes, and rock cervices up to 1500 m above mean sea level (Chauhan 1999; Upadhyay *et al.* 2011). In Pakistan it is found in northern hilly areas, where in local Hindi/Punjabi language it is called kori booti (means bitter herb) owing to its bitter taste. It is found along roadsides, open slopes, and rock cervices. The plant is used for the treatment of gout, rheumatism, palsy and amenorrhoea. Locally the leaves help in curing headache, pimples, measles, stomach acidity, burns, boils. It is effectively used against jaundice, hypertension, sore throat and as a blood purifier.

Materials and Methods

Collection and air drying of plant material: Aerial parts of *Ajuga bracteosa* were collected from Sinthan Top area of District Anantnag (Kashmir) in the month July, 2013. The plant was identified at the Centre of Biodiversity and Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar, J and K and a voucher specimen (JKASH/CBT/226; Dated 08. 08. 2014) was deposited there. The parts were allowed to dry under shade (30 °C) for 8-10 days.

Preparation of extracts: After shade drying, the aerial parts were macerated to fine powder, 1 kg of leaves were extracted successively with hexane for defatenning and methanol for 16 h using Soxhlet apparatus. The extracts were filtered through a Buchner funnel using Whatman No. 1 filter paper, and all the extracts were concentrated to dryness under vacuum using a Heidolph rotary evaporator, yielding hexane, ethyl acetate, methanol and aqueous crude extracts of 65, 52, 46 and 36 g respectively. All the extracts were stored at 4°C in air tight glass bottles before use.

Phytochemical screening: Chemical tests were carried out on the extracts using standard procedures to identify the constituents (Harborne 1984; Evans 1989; Sofowora, 1993; Okwu 2004).

Test for tannin: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue- black colouration.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoid.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar 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.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
b) **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
c) **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
d) **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
b. **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
c. **Fehling's Test:** Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Phytosterols

a. **Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
b. **Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols: Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of proteins and amino acids

a. **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
b. **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Results and Discussion

Therapeutic values of medicinal and aromatic plants (MAPs) are due to the presence of major bioactive constituents like alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, saponins, steroids etc. The phytochemical investigation of *Ajuga bracteosa* extracts in the present study revealed presence of different active ingredients (secondary plant metabolites) like flavonoids, phenolics, alkaloids, tannins, cardiac glycosides, terpenes, saponins, steroids, carbohydrates, amino acids and proteins as shown in Table 1. It supports the resourcefulness of the plant extract (Sofowora, 1993).

Table 1: Qualitative phytochemical screening of *Ajuga bracteosa*

Phytoconstituents	Test	Result
Alkaloids	Wagner's test	+ +
Phenolics	phenol test	+ +
Tannins	Ferric chloride test	+ +
Cardiac glycosides	Keller-Killani test	+ +
Terpenes	Salkwaski's test	+
Flavonoids	Shinoda's test	+ +
Saponins	Frothing test	+
Steroids	Libermann-Buchard's test	+
Carbohydrates	Molish test	+ +
Proteins	Biuret test	+
Polysterols	Salkowski's Test	+
Amino acids	Ninhydrin Test	+

(++) = strong presence, (+) = moderate presence

From ancient times, medicinal plants are being used as remedies for various diseases in human. In today's industrialized society, the use of medicinal plants has been traced to the extraction and development of several drugs as they were used traditionally in folk medicine (Shrikumar and Ravi 2007). Medicinal plants have potent phytoconstituents which are important source of antibiotic compounds and are responsible for the therapeutic properties (Jeeva *et al.* 2011; Jeeva and Johnson 2012; Florence *et al.* 2012, 2014; Joselin *et al.* 2012, 2013; Sainkhediya and Ray 2012; Sumath *et al.* 2014). These phytoconstituents endow them with medicinal properties. Many plants possess antioxidant properties because of the presence of phenolic compounds (Brown and Rice-Evans 1998; Krings and Berger 2001). These phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.* 2007). Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie 1996). They also are effective antioxidant and show strong anticancer activities (Salah *et al.* 1995; Del-Rio *et al.* 1997).

Besides, most of the phytochemicals are known to have therapeutic properties such as insecticidals (Kambu *et al.* 1982), antibacterial, antifungal (Lemos *et al.* 1990) and anticonstipative (Ferdous *et al.* 1992) activities etc. The plants thus find their medicinal values due to the presence of these phytochemical constituents. The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.

Ajuga bracteosa also contain saponins which are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000). Steroids, another important phytoconstituent present in Ajuga bracteosa, have been reported to possess antibacterial properties (Raquel 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al.* 1994). It has been reported that alkaloids possess analgesic (Antherden, 1969), antispasmodic and antibacterial (Stray, 1998; Okwu and Okwu, 2004) properties. Glycosides are known to lower the blood pressure according to

many reports (Nyarko and Addy 1990). Thus from the present study, it could be suggested that the identified phytoconstituents from *Ajuga bracteosa* make the plant valuable for bioactive compounds of sustainable medicine.

Conclusion

The medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Thus, *Ajuga bracteosa* can be used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. Thus we hope that the important phytochemical properties identified in this study in the local plant of Kashmir Himalaya will be helpful in copping different diseases of this particular region.

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