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A COMPARATIVE ANALYSIS OF THE PHYTOCHEMICAL CONSTITUENTS OF TWO SPECIES OF *AJUGA* L

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ABSTRACT

Plants are bestowed with various chemical constituents like phenols and flavonoids that possess therapeutic and medicinal importance. Such phytochemical constituents may be considered as an alternative means for synthetic medicine because of low toxicity. These secondary metabolites are being extensively investigated as a source of medicinal agents. Ajuga bracteosa and Ajuga parviflora are highly valuable medicinal plants growing in Kashmir Himalaya. The present study was conducted to determine the qualitative analysis of the crude extracts of A.bracteosa and A.parviflora. The extracts were prepared by using Petroleum ether, Ethyl acetate, Methanol and Aqueous solvents. These plant extracts were found positive for a wide range of bioactive compounds. The study revealed the presence of alkaloids, phenolics, flavonoids, tannins, terpenoids and saponins in the crude extracts of two Ajuga species .The methanolic extracts of both the species of Ajuga were further analysed for the quantitative estimation of these secondary metabolites.The Quantitative analysis of the methanolic extracts revealed total phenolic content of 9.56±0.55 mgGAE/g and 10.16±0.08 mgGAE/g for A.bracteosa and A.parviflora respectively. The flavonoid content was 6.11±0.64 mg Rutin/g and 6.21±0.28 mg Rutin/g for A.bracteosa and A.parviflora respectively. A fairly good content of saponins, terpenoids and alkaloids was also found in the methanolic extracts of these two Ajuga species. Hence the two plant species under study may prove as source of various phytochemical constituents with potent medicinal activity.

Keywords: Ajuga bracteosa, Ajuga parviflora, Phytochemical Analysis, qualitative analysis, quantitative analysis, phenols and flavonoids.

I. INTRODUCTION

Phytochemical constituents are biologically active, naturally occurring chemical compounds found in plants, which protect plants from disease and damage and contribute to the plant's colour, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (1; 2). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. The qualitative and quantitative

estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research (3). These phytochemicals, particularly phenols exhibit a wide variety of actions like anti-bacterial, immune- stimulating, anti-allergic, anti-hypertensive, anti-ischemic, anti- thrombotic, hypochollesterolemic, anti-lipoperoxidannt, hepatoprotective, anti- inflammatory and anti-carcinogenic (4). Phytochemical constituents are effective in the treatment of various diseases while simultaneously mitigating many of the side effects that are often associated with synthetic medicine (5). In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action, eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant. The therapeutic actions of plants are unique to particular plant species (6).

Ajuga bracteosa belongs to genus *Ajuga* L. and family *Lamiaceae* (the largest family of order Lamiales). It is distributed in sub-tropical and temperate regions from Kashmir to Bhutan, Pakistan, Afghanistan, China and Malaysia (7). *Ajuga parviflora* Benth. is a small perennial herb growing wild in the temperate regions of Himalaya, Afghanistan, Kashmir and Pakistan (8; 9).Both these species have been found growing in Kashmir region. The two species, due to their resemblances are both locally known as *Jan e adam*.The main objective of the present study was to screen, evaluate and compare the whole herbs of these two species of genus *Ajuga* for their phytochemical constituents rendering potent medicinal activities (antimicrobial, antioxidant and anticancerous) to these two species.

II. MATERIAL AND METHODS

2.1 Plant material

Fresh whole herbs of *Ajuga bracteosa* and *Ajuga parviflora* were collected judiciously from different sites of Kashmir valley in the month of May- June 2017. The plant material was properly identified by Dr. Irshad A. Nawchoo, Professor, Department of Botany, University of Kashmir (Srinagar, India). A voucher specimen was deposited in Kashmir University Herbaria (KASH) for further reference.

2.2 Preparation of extract

The collected herbs were cleaned and cut into small pieces and then air dried under shade at room temperature (25°C). The dried material was ground to fine powder using a mechanical blender and powdered plant material (50 g) was packed in Soxhelt apparatus and extracted with Petrolem ether, ethyl acetate and methanol and aqueous solution successively. The extract was filtered through Whatmann filter paper No.1 and the solvent was removed under reduced pressure at 35-45°C using rotavapor. The dried extract was labelled and stored at 4°C in air tight glass bottles before experimental use.

2.2 Qualitative Phytochemical Screening

Phytochemical screening for major bioactive constituents like alkaloids, phenolics, flavonoids and tannins were determined by using standard phytochemical methods.

- ***Alkaloids (Wagner's test):*** To confirm the presence of alkaloids, 1.5 ml of HCl (1%) was added to 2 ml of Methanolic extract filtrate. The reaction mixture was heated in a water bath, followed by addition of six drops of Wagner's reagent. Orange precipitate formation at the bottom of test tube confirmed the presence alkaloids (10).
- ***Phenolics (Phenol test):*** For detection of phenolics, 1 ml of $FeCl_3$ (1%) was added to 2 ml of alcoholic extract. Formation of blue or green colour was the indication of presence of phenolics (11).
- ***Tannins (Ferric chloride test):*** 2 ml of $FeCl_3$ (5%) was added in 2 ml of alcoholic extract. Yellow brown precipitate formation at the bottom of test tube confirmed the presence tannins (12).
- ***Terpenes (Salkowski's test):*** Chloroform (5 ml), acetic anhydride (2 ml) and few drops of Conc. H_2SO_4 were added to 2 ml of alcoholic extract with care. Formation of reddish brown ring at interface confirmed the presence of terpenes (13).
- ***Flavonoids (Shinoda's test):*** In 2 ml of alcoholic filtrate, few drops of Conc. HCl was added followed by addition of pinch of zinc or magnesium turnings. Formation of magenta red or pink colour confirmed the flavonoids presence (10, 11).
- ***Saponins (Frothing test):*** Alcoholic extract was subjected to frothing test. Persistence of frothing confirms saponins presence. Latter few olive oil drops were added. Emulsion formation indicated the presence of saponins (13, 14).
- **Quinones (Hydrochloric acid test):** A small amount of extract was treated with concentrated hydrochloric acid and observed for the formation of yellow precipitate or colouration for the presence of quinones (15).

2.3. Quantitative estimation

- **Determination of total phenolics**

The total phenolic content was determined using a modified spectrophotometric method (16). The reaction mixture was prepared by mixing 0.5 mL of methanol solution (1 mg/mL) of each extract with 2.5 mL of 10% Folin-Ciocalteu reagent dissolved in water and 2.5 mL of 7.5% $NaHCO_3$. The samples were incubated at 45°C for 15 min. The absorbance was measured at λ_{max} = 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. A blank was concomitantly prepared with methanol instead of extract solution. The same procedure was repeated for the gallic acid, and the calibration curve was constructed. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GAE/g of extract**).**

- **Determination of total flavonoids**

The concentration of flavonoids was determined using a spectrophotometric method with some modifications (17). The sample contained 1 mL of methanol extract in a concentration of 1 mg/mL and 1 mL of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for 1 h at room temperature. The absorbance was

measured at λ_{max} = 415 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. In- stead of extract solution, methanol was used in order to prepare a blank. The same procedure was repeated for rutin, and the calibration curve was constructed. Concentration of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

- **Saponin estimation**

  For the determination of saponins, 10g of each plant sample was weighed, and was dispersed in 100ml of 20% aqueous ethanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55°C. The extract was concentrated to 40 ml over water bath at 90°C. Concentrate obtained was transferred into a separating funnel and 10 ml of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ethyl layer was discarded. The process was repeated. To the aqueous layer n-Butanol was added. The whole mixture was washed in separating funnel twice with 10 ml 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Later it was dried in oven to a constant weight and the saponin content was calculated (18).The values were presented as mean ± SD of triplicate analysis.

- **Determination of terpenoids**

50g of plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids (19).

- **Alkaloid estimation**

2.5g of the plant powder was extracted using 100ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25ml. Concentrated ammonium chloride was added stepwise for precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed (18,20).

III. RESULTS AND DISCUSSION

The phytochemical investigation of whole plant extracts of *Ajuga bracteosa* and *Ajuga parviflora* revealed strong to moderate presence of different bioactive ingredients (secondary plant metabolites) like alkaloids, phenolics, flavonoids, tannins, terpenoids and saponins as shown in (**Table 1 and Table 2**). The medicinal and aromatic plants (MAPs) have therapeutic values due to the presence of these major bioactive constituents. The Quantitative analysis of the methanolic extracts revealed total phenolic content of 9.56±0.55 mg GAE/g and 10.16±0.08 mg GAE/g for *A.bracteosa* and *A.parviflora* respectively. The flavonoid content was 6.11±0.64 mg Rutin/g and 6.21±0.28 mg Rutin/g for *A.bracteosa* and *A.parviflora* respectively.A fairly good content of saponins, terpenoids and alkaloids was also found in the methanolic extracts of the *Ajuga* species (**Table 3; Fig 1**).

The medicinal and aromatic plants (MAPs) have therapeutic values due to the presence of major bioactive

constituents like alkaloids, phenolics, flavonoids, tannins, terpenes, saponins. It supports the resourcefulness of the plant extracts (13). Preliminary phytochemical investigation of different extracts of whole plants of *Ajuga bracteosa* and *Ajuga parviflora* extracts revealed strong to moderate occurrence of variety of plant bioactive metabolites such as alkaloids, phenolics, flavonoids, tannins, terpenes, saponins. Phenolic compounds in the plants indicate that they may possess anti-microbial properties. Our studies corroborate with the findings of Ofokansi *et al*.,2005 (21). Phenolic compounds possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (22). Flavonoids, on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (20). Saponins may be considered a part of plants' defence systems, and possess diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals Saponin also have the property of precipitating and coagulating red blood cells (23,20). Terpenoids can have medicinal properties such as anti- carcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol (24). Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine). These are just a few examples illustrating the great economic importance of this group of plant constituents (25).

IV. CONCLUSION

The present study has provided the chemical basis for the wide use of *A.bracteosa* and *A.parviflora* as therapeutic agents for treating various ailments.The secondary metabolite content is approximately similar in both the species thus rendering tremendous medicinal importance to both these species. However, there is need to further carry out advanced studies in order to elucidate the structure of these compounds.

Table 1: Qualitative phytochemical screening of whole plant extracts of *Ajuga bracteosa*

Phytoconstituents	Test	Aqueous	Methanoli	Ethyl	Petroleum
Alkaloids	**Wagner's test**	+	++	++	+
Phenolics	**Phenol test**	+	++	+	-
Tannins	**Ferric chloride test**	+	++	+	_
Terpenes	**Salkwaski's test**	+	++	+	+
Flavonoids	**Shinoda's test**	+	++	+	-
Saponins	**Frothing test**	+	++	-	-
Quinones	**HCl test**	+	++	++	+

(++), strong presence, (+), moderate presence, (-) absent

Table 2: Qualitative phytochemical screening of whole plant extracts of *Ajuga parviflora*

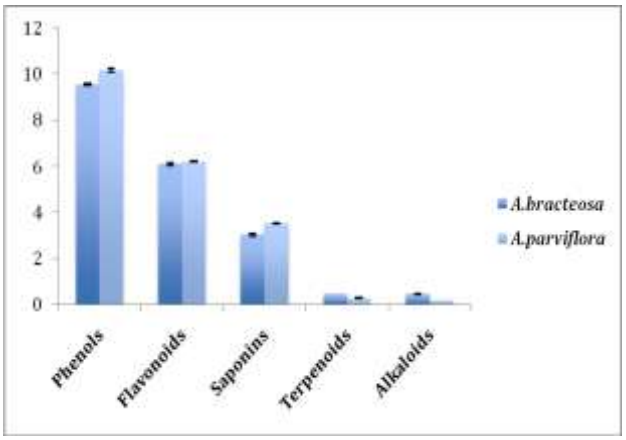
Phytoconstituents	Test	Aqueous Extract	Methanolic Extract	Ethyl Acetate Extract	Petroleum ether extract
Alkaloids	**Wagner's test**	+	++	+	+
Phenolics	**phenol test**	+	++	+	-
Tannins	**Ferric chloride test**	-	++	-	-
Terpenes	**Salkwaski's test**	-	++	+	+
Flavonoids	**Shinoda's test**	+	++	+	-
Saponins	**Frothing test**	+	++	-	-
Quinones	**HCl test**	+	++	-	-

(++), strong presence, (+), moderate presence, (-) absent

Table 3: Quantitative estimation of phytochemicals of the methanolic extracts of *A.bracteosa* and *A.parviflora.*

Name of the species	Phytochemical Constituents				
	Phenols	**Flavonoids**	**Saponins**	**Terpenoids**	**Alkaloids**
Ajuga brateosa	9.56±0.55	6.11±0.64	3.03±0.06	0.48±0.02	0.46±0.01
Ajuga parviflora	10.16±0.08	6.21±0.28	3.53±0.36	0.29±0.01	0.18±0.01

Fig 1. Figure showing a comparison in the secondary metabolite content of *A.bracteosa* and *A.parviflora.*



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