

Sterol composition of *Ajuga bracteosa* Wall Benth., *Ajuga macrosperma* Wall ex Benth. and *Ajuga parviflora* Benth.

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Abstract : Sterols isolated from *Ajuga bracteosa* Wall Benth., *Ajuga macrosperma* Wall ex Benth. and *Ajuga parviflora* Benth. of family Lamiaceae were studied by GC-MS analysis of their trimethylsilyl ether derivatives. Individual sterols and conjugated authentic samples were used for identification of components present in the sterol mixtures isolated from the plants. *A. bracteosa* Wall Benth. was found to contain the highest amount of dehydroclerosterol (51.14%) followed by clerosterol (36.18%) of the total sterol content. Campesterol (2.30%), sitosterol (1.27%) was present in lower quantities. *A. macrosperma* Wall ex Benth. contained dehydroclerosterol (58.89%), clerosterol (35.58%), sitosterol (1.89%) and campesterol (1.73%). *A. parviflora* Benth. contained clerosterol (58.71%), dehydroclerosterol (37.80%), sitosterol (0.93%) and campesterol (0.76%). Stigma sterol could not be detected in any samples.

Keywords : Lamiaceae, *Ajuga bracteosa*, *Ajuga macrosperma*, *Ajuga parviflora*, sterols.

Introduction

Genus *Ajuga* has great medicinal and economic importance *Ajuga bracteosa* Wall Benth., *Ajuga macrosperma* Wall ex Benth. and *Ajuga parviflora* (Common name : Small-Flowered Bugleweed) are small short lived perennial herbs native to Asia-temperate and Asia-tropical regions and grows in shady areas in forests, ravines, grassy roadsides at an altitude of 400–2600 m¹, which grow wild in Uttarakhand region of western Himalaya. Plants of genus *Ajuga* produce variety of biological active. Secondary metabolites including phytoecdysteroids, neoclerodane diterpenoids, iridoids, sterols, withanolids, anthocyanins, flavonoids, ionones, quinones and have been used in folk medicine because of their anthelmintic, antifungal, hypoglycemic, antitumor, and antimicrobial properties^{2,3}. Phytosterols also referred to as plant sterol and stanol esters are steroid compounds similar to cholesterol found in plants and vary only in carbon side chains and or presence or absence of a double bond. Since, phytosterols are structurally similar to the cholesterol; they compete with it for absorption in the digestive system when consumed. As a result, cholesterol absorption is blocked, and blood cholesterol level is reduced. Sitosterol, campesterol and stigmasterol are most abundant in nature comprising 65%, 30% and 3% of dietary phytosterol in-

take. Other common phytosterols and phytostanols are sitosteranol, campestanol and brassicasterol⁴. When reviewing clinical trials involving phytosterol supplementation, the FDA concluded that when consumed in the range of 1 to 3 grams in enriched foods, phytosterols resulted in statistically significant (5–15%) reductions in blood LDL cholesterol levels relative to placebo⁵. No previous studies have been reported on phytosterol composition of *Ajuga* species growing in Himalayan region. We report the sterol composition of *A. bracteosa*, *Ajuga parviflora* and *A. macrosperma*.

Experimental

Fresh plants of *Ajuga bracteosa*, *Ajuga macrosperma* and *Ajuga parviflora* were collected, shade dried and powdered. The shade dried powdered plant materials of *Ajuga bracteosa*, *Ajuga macrosperma* and *Ajuga parviflora* were subjected to soxhlet extraction using methanol as solvent. The methanolic extract of *A. macrosperma* (AMME) was dried under vacuum. This was re-dissolved in methanol : water (1 : 1) and then fractionated successively partitioning with *n*-hexane, dichloromethane, ethyl acetate and butanol. The extracts were filtered individually and the solvents were evaporated using thin film vacuum rotatory evaporator. The extracts were kept in

refrigerator for further use.

Isolation of phytosterols from *Ajuga* species :

The hexane extracts (10 g) of aerial parts of *Ajuga parviflora*, *Ajuga macrosperma* and *Ajuga bracteosa* were loaded separately on 100 g silica (60–120 mesh, BDH) columns pre-packed in hexane. The columns were first eluted with hexane and then hexane ethyl acetate mixtures. Hexane 80 : 20 fraction gave single spot on TLC. The solvent was evaporated under vacuum and the fraction were re-crystallized with methanol : ether. This gave fine needles of sterol mixture (~20–30 mg) which were further subjected to GC-MS analysis.

Preparation of TMS derivatives :

The purified phytosterols 100 mg were dissolved in methylene chloride (100 μ L) and placed in capped test tubes. Trimethylsilyl (TMS) ether reagent (200 μ L) was added to each and the test tube heated at 60 °C for 20 min in a heating block after tightly capping. The reagent was removed under nitrogen gas, then was dissolved in 250 μ L methylene chloride and stored in a freezer for GC and GC-MS analysis.

GC/MS analysis :

GC-MS analysis was performed using gas chromatograph HP 6890 with mass selective detector MS 5973 (Agilent Technologies, USA) fitted with a HP-5MS fused silica column (30 m \times 0.25 mm; 0.25 μ m film; thickness), with electronic pressure control and split-split less injector. Helium flow rate through the column was 1 mL/min in a constant flow mode. The initial column temperature was 50 °C rising 250 °C at a rate 5 °C/min. The MS detector acquisition parameters : transfer line held at 260 °C and detector was held at 280 °C. Detection was performed in full scan mode from m/z 41 to 450.

Identification of sterols :

The identification of trimethylsilyl derivatives from the purified phytosterols was done by comparison of relative retention times and mass spectra of samples with those of TMS derivatives of authentic standards.

Results and discussion

Sterols isolated from *Ajuga bracteosa* Wall Benth., *Ajuga macrosperma* Wall ex Benth. and *Ajuga parviflora* Benth. were analyzed by GC-MS analysis of their trimethylsilyl (TMS) ether derivatives. Individual sterols and conjugated authentic samples were used for identification of components present in the sterol mixtures iso-

lated from the plants. *Ajuga bracteosa* Wall Benth. (ABT) contained the highest amount of dehydroclerosterol (51.14%) followed by clerosterol (36.18%) of the total sterol content. Campesterol (2.30%), sitosterol (1.27%) was present in lower quantities. *Ajuga macrosperma* Wall ex Benth. (AMP) contained dehydroclerosterol (58.89%), clerosterol (35.58%), sitosterol (1.89%) and campesterol (1.73%). *Ajuga parviflora* Benth. (AVP) contained clerosterol (58.71%), dehydroclerosterol (37.80%), sitosterol (0.93%) and campesterol (0.76%). Stigma sterol was absent in all the samples. Plant sterols are used to lower the serum cholesterol levels by decreasing the cholesterol absorption in plant sterols⁷. Moreover, sterols are precursor of phytoecdysteroids. Clerosterols and dehydroclerosterol are known precursors for phytoecdysteroids^{6,7}. In earlier report from our group, phytosteroids have been reported from *Ajuga macrosperma*⁸. Only β -sitosterol have also been reported from *Ajuga bracteosa*⁹, however presence of phytoecdysteroids has not yet been reported from *A. parviflora* and *A. bracteosa*. The presence of cholesterol and dehydrocholesterol in *A. parviflora* and *A. macrosperma* indicate the possibility of presence of phytosterols in these two species.

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References

1. R. Strachey, "The catalogue of the plants of Kumaon and portions of Garhwal and Tibet", Periodical Expert Book Agency, New Delhi, 1974.
2. J. Cou and Y. A. Tandon, *Phytochem. Rev.*, 2008, **7**, 25.
3. Israili, H. Zafar and L. Badiia Pak, *J. Pharm. Sci.*, 2009, **22**, 425.
4. J. L. Weihrauch and J. M. Gardner, *J. Am. Diet. Assoc.*, 1978, **73**, 39.
5. J. Saji, A. V. Sorokin and P. D. Thompson, *Current Opinion in Lipidology*, 2007, **18**, 35.
6. K. Okuzumi, H. Noriyuki, F. Yoshinori, Y. Junko, N. Atsuko, T. Kyoko and M. Masuo, *Tetrahedron Lett.*, 2003, **44**, 323.
7. K. Okuzumi, H. Noriyuki, U. Hidehiro and F. Yoshinori, *Biomol. Chem.*, 2005, **3**, 1227.
8. A. Castro, J. Coll, Y. A. Tandón, A. K. Pant and C. S. Mathela, *J. Nat. Prod.*, 2008, **71**, 1294.
9. V. H. Verma, U. Mahmood and B. Singh, *Nat. Prod. Lett.*, 2002, **16**, 255.