Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Immunostimulant Activity of *Bergenia* Extracts

Lenka Tumová, Helena Hendrychová, Doris Vokurková[1]

Department of Pharmacognosy, Faculty of Pharmacy Hradec Králové, Charles University, [1]Departmen of Clinical Immunology and Allergology, Faculty of Medicine and University Hospital, Charles University, Sokolská, Hradec Králové, Czech Republic

Submitted: 13-09-2017 Revised: 20-10-2017 Published: 14-08-2018

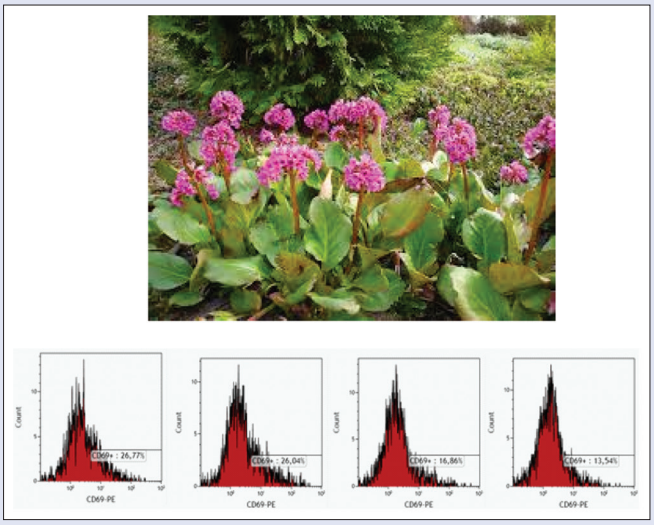
ABSTRACT

Background: *Bergenia* species contain various therapeutically important compounds such as phenolic compounds-arbutin, bergenin, tannins, gallic acid, flavonoids, minerals, and many others. *Bergenia* plants show antibacterial, antiviral, anticancer, antidiabetic, diuretic and immunostimulant activities. **Materials and Methods:** *Bergenia* leaf extracts from *Bergenia crassifolia*, *Bergenia ciliata*, and *Bergenia* x *ornata* on lymphocyte activation using flow cytometry method were investigated. Their activation was then monitored with the help of the increasing fluorescence intensity. **Results:** By activating of cells, there has been an increase in the amount of the established antibodies. *Bergenia* extracts significantly stimulated the expression of CD69 on lymphocytes in the final concentration of 3.13 and 6.25 mg/ml. The values of stimulation indices of *B. crassifolia* and *B.* x *ornata* extract significantly statistically did not differ. **Conclusion:** The values of stimulation indices of *B. crassifolia* and *B. x ornata* extract significantly statistically did not differ.

Key words: *Bergenia*, immunostimulant activity, lymphocytes

SUMMARY

- The *ex-vivo* activation of human immune cells, as determined by cell surface CD69, was reported for the first time in Bergenia extracts.



Correspondence:

Dr. Lenka Tumová,
Department of Pharmacognosy,
Faculty of Pharmacy Hradec Králové,
Charles University, Heyrovského 500 05, Hradec Králové, Czech Republic.
E-mail: tumova@faf.cuni.cz
DOI: 10.4103/pm.pm_423_17

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Bergenia genus belongs to *Saxifragaceae* family is one of the very important medicinal plants. Over 30 species of this genus is known at present[1] in the world. Therapeutically, interesting compounds are present not only in roots, stems but also in leaves of this plant. Due to the content of various primary and secondary metabolites, many biological activities were found there. *Bergenia* contains polyphenols such as arbutin, bergenin, catechin, gallic acid, acylated gallic compounds, (+)-afzelechin, and leukocyanidin. Other bioactive ingredients are also flavonoids (kaempferol and quercetin), quinones (aloe-emodin, physcion, and hydroquinone) and others (volatile oils, polysaccharides, carotenoids, amino acids, sterols, and mineral elements).[2-4] *Bergenia* plants show antibacterial, antiviral, anticancer, antidiabetic, diuretic and immunostimulant activities.[5-10] Leaf extracts of *Bergenia* plants on the specific immune response was tested in mice *in vivo* (with a minimum amount of arbutin around 18%). Extracts have led to the normalization of the content of immunocompetent cells in the spleen of the test animals. The extracts decreased the manifestation of the inflammatory process by preventing the accumulation of lymphocytes and a reduction in the ability of cells to produce cytokinins.[10] Polysaccharide bergenan was isolated from the freshly collected green leaves of *Bergenia crassifolia*. Its administration in the form as the solution to mice (2 mg/ml) for 3 weeks showed an increase in the immune response.[11,12]

Immunostimulant activity

Transmembrane glycoprotein CD69 is the current activating antigen arranging timely transmembrane signals. Maximal expression achieves already after 18–24 h after stimulation. CD69 has a great importance in the immune response.[13]

Cite this article as: Tumová L, Hendrychová H, Vokurková D. Immunostimulant activity of *Bergenia* extracts. Phcog Mag 2018;14:328-32.

In this study, the effect of lyophilized leaf ethanol *Bergenia* extracts (BCR 2, BCL 2, and BOR 1) on lymphocyte activation by the flow cytometry method was investigated. By activating of cells, there has been an increase in the amount of the established antibodies. Their activation was then monitored with the help of the increasing fluorescence intensity.

MATERIALS AND METHODS

Plant material

Leaves of *B. crassifolia* L. (Fritsch.), *Bergenia ciliata* Sternb., and *Bergenia* x *ornata* were obtained from the Botanical Garden of Faculty of Horticulture, Mendel University, Brno.

Method

Immunostimulant activity

This method modified according to the work of the Cheel[14] monitored the impact of leaf lyophilized ethanol extracts of *B. crassifolia* (BCR 2), *B. ciliata (*BCL 2), and *B.* x *ornata* (BOR 1) on the activation of the CD69 cells using flow cytometry. Testing was conducted on a 96-hole microtiter plate. For testing, the peripheral blood with heparin from three healthy donors was used. A volume of 100 µl of this blood was incubated with 100 µl of X-VIVO™ media and with the tested extracts diluted by the growing concentration and control. Extracts were prepared from 0.8 mg lyophilizate with 10 µl of DMSO (concentration in the solution to 0.5%) and 1 ml X-VIVO™ medium. As blank a solution of 100 µl X-VIVO™ medium with 100 µl of blood was used. The positive control was prepared from 100 µl of blood and 100 µl of PHA (phytohemaglutinin in the concentration of 10 µg/ml X-VIVO™ media). As a control, 0.5% solution of DMSO was also prepared. The samples before the actual analysis were filtered through a microfilter (0.2 µm). The final concentration of the tested substances was in the range of 0.2–0.8 mg/ml and 2–50 mg/ml. The microtiter plates were incubated at 37°C for 24 h in the presence of 5% CO_2. After the incubation, 40 µl of the suspension was stirred with fluorescent-unstable antibodies (CD69 PE). The analysis was used by flow cytometer Cytomics FC500 (Beckman Coulter), data were analyzed using the CXP analytical software (Coulter Electronic). Activation of lymphocytes (90% T-cells) was tracked with the help of the increasing mean fluorescence intensity (MFI), which was caused by the expression of CD69 on the cell surface. Fluorescent signals were recorded logarithmically as the amplified signals. The values of activation were expressed as activation indexes (AI), which were obtained by comparing the fluorescence intensities of the test samples and the control and stimulant indexes (SI) corresponding to the ratio of the values of the activated sample to the negative

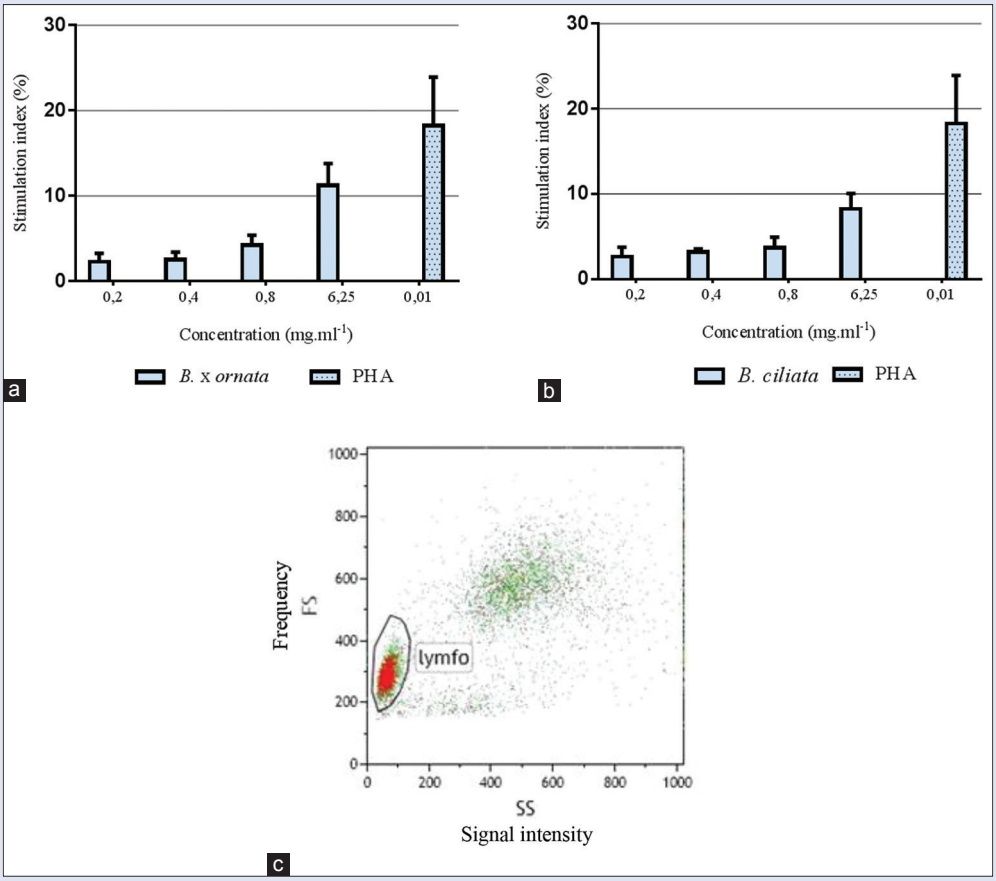


Figure 1: The effect of *Bergenia* extracts at the final concentration of 0.20–6.25 mg/ml *Bergenia* x *ornata* (a) and *Bergenia ciliata* (b) on the activation of lymphocytes corresponding to the expression of CD69. Because of the elimination of the donors variability the resulting values are expressed using the stimulus indices (%). As a positive control phytohemaglutinin was used. Values are the average of three experiments ± standard deviation. Lymphocytes were identified on the basis of the direct scattering (FS) and side scattering (SS). Activated cells (lymphocytes) have been identified in the circulate gate (c)

control (eliminating thus the variability of the donors). A positive immune cellular response was defined as AI ≥2.[14] The measurement of the immunostimulant activity of selected *Bergenia* plant extracts conducted at the Institute of Clinical Immunology and Allergology at University Hospital in Hradec Králové.

Statistical analysis

Significant differences between values were determined by one–way analysis of variance ANOVA. For determining pair-wise differences of means, the Tukeyś test was performed, $P \leq 0.05$). Data are presented as the means ± standard deviation of three experiments.

RESULTS AND DISCUSSION

Bergenia extracts significantly stimulated the expression of CD69 on lymphocytes in the final concentration of 3.13 and 6.25 mg/ml. On the contrary, higher concentration of extracts of 12.5; 25 and 50 mg/ml were toxic for the cell and they so did not stimulate the expression of CD69. Low concentration (0.2–0.8 mg/ml) was on the contrary too weak to stimulate the expression of CD69 [Figure 1]. The values of stimulation indices of *B. crassifolia* [Figure 2a] and *B. x ornata* [Figure 2b] extracts significantly statistically did not differ.

The highest activation of lymphocytes reached the *B.* x *ornata* extract at a concentration of 6.25 mg/ml [Figures 2b and 1a]. The histograms in Figure 3 depict a statistically significant stimulation of the CD69 expression on lymphocytes using leaf *Bergenia* extracts in concentrations of 1.56–6.25 mg/ml, compared to a blank sample (X-VIVO™ medium) and positive control (PHA).

As is apparent from the literature, there is so far no study compared the effect of *Bergenia* extracts on the activation of lymphocytes. As reported by Popov *et al.*,[15] a profound immunostimulant effect and fagocytic activity exhibit a polysaccharide bergenan isolated from the leaves of *B. crassifolia*. In the work of the Nazir *et al.*,[16] immunomodulatory effect of bergenin and norbergenin on TH1 (inflammation TH cells capable of direct activities) and TH2 (activation of B-lymphocytes) there have been shown.

CONCLUSION

The *ex vivo* activation of human immune cells, as determined by cell surface CD69 expression, was reported for the first time in *Bergenia* extracts. By activating of cells, there has been an increase in the amount of the established antibodies. *Bergenia* extracts significantly stimulated the expression of CD69 on lymphocytes in the final concentration of 3.13 and 6.25 mg/ml. The values of stimulation indices of B. *crassifolia* and *B.* x *ornata* extracts significantly statistically did not differ. The values of stimulation indices of *B. crassifolia* and *B.* x *ornata* extract significantly statistically did not differ.

Financial support and sponsorship

This work was supported by SVV 260416.

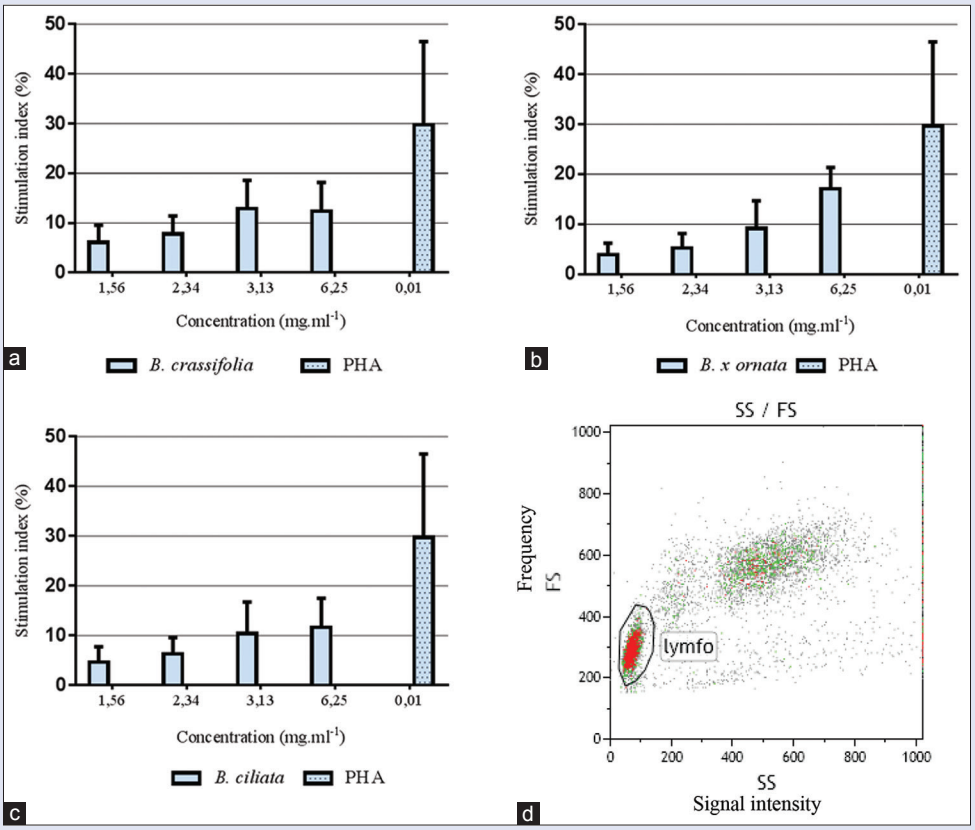


Figure 2: The effect of the *Bergenia* extracts at the final concentration of 1.56–6.25 mg/ml *Bergenia crassifolia* (a), *Bergenia* x *ornata* (b) and *Bergenia ciliata* (c) on the activation of lymphocytes corresponding to the expression of CD69. Because of the elimination of the donors variability, the resulting values are expressed using the stimulus indices (%). As a positive control phytohemagglutinin (PHA) was used. Values are the average of three experiments ± standard deviation. Lymphocytes were identified on the basis of the direct scattering (FS) and side scattering. Activated cells (lymphocytes) have been identified in the circulate gate (d)

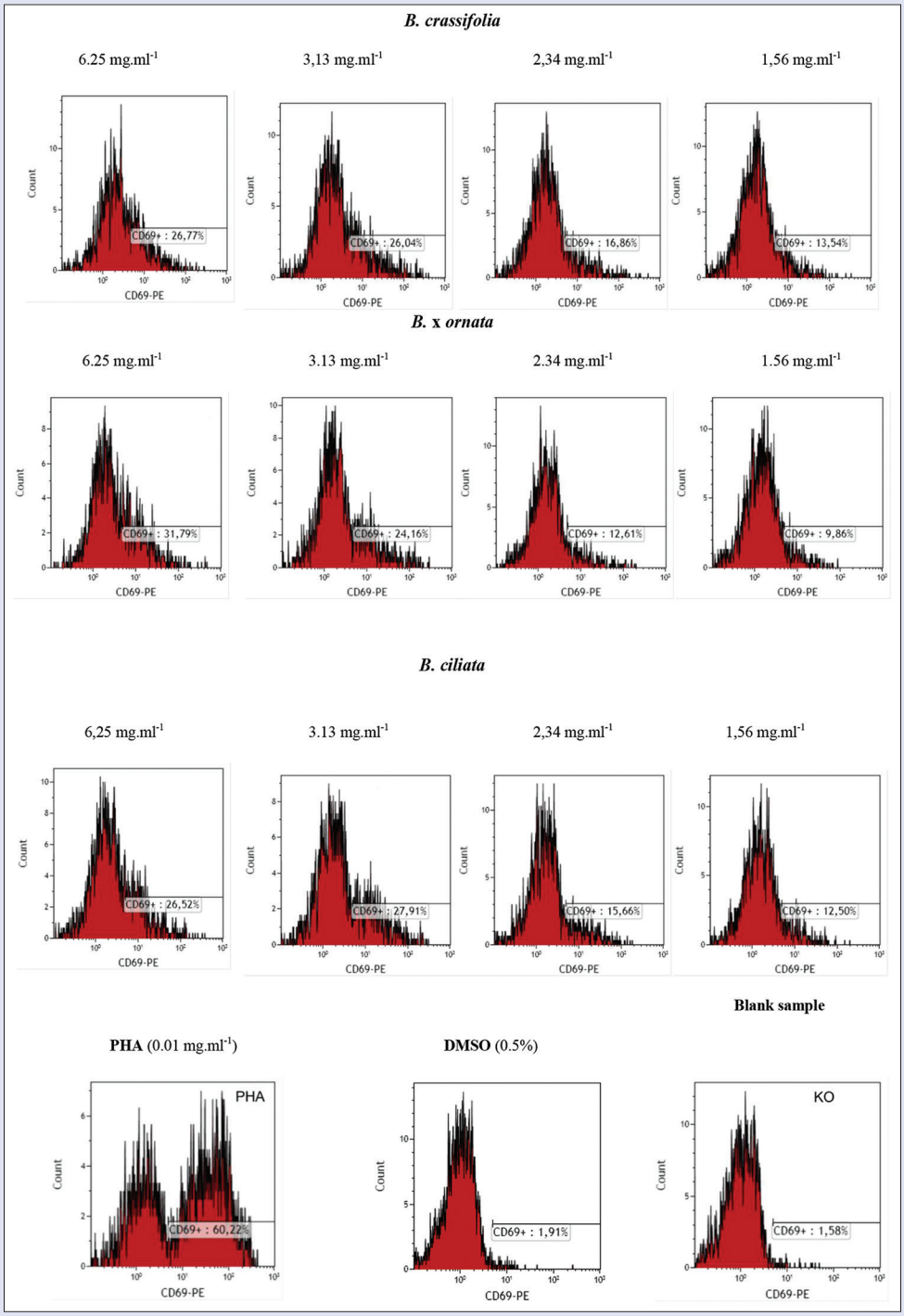


Figure 3: The dependence of lymphocyte activation on the concentration of ethanol *Bergenia* extracts (1.56–6.25 mg/ml). Histograms capture the expression of CD69 (%) on the surface of lymphocytes as compared to positive control (PHA), a blank sample (X-VIVO™ medium) and DMSO

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Zhang Y, Liao C, Li J, Liu X. A review on resource status, bioactive ingredients, clinical applications and biological progress in *Bergenia*. J Med Plants Res 2011;5:4396-99.
2. Salminen JP, Shikov AN, Karonen M, Pozharitskaya ON, Kim J, Makarov VG, *et al.* Rapid profiling of phenolic compounds of green and fermented *Bergenia crassifolia* L. Leaves by UPLC-DAD-qqQ-MS and HPLC-DAD-ESI-QTOF-MS. Nat Prod Res 2014;28:1530-3.
3. Chernetsova ES, Shikov AN, Crawford EA, Grashorn S, Laakso I, Pozharitskaya ON, *et al.* Characterization of volatile and semi-volatile compounds in green and fermented leaves of *Bergenia crassifolia* L. by gas chromatography-mass spectrometry and id-cube direct analysis in real time-high resolution mass spectrometry. Eur J Mass Spectrom (Chichester) 2014;20:199-205.
4. Shikov AN, Pozharitskaya ON, Makarova MN, Makarov VG, Wagner H. *Bergenia crassifolia* (L.) fritsch – Pharmacology and phytochemistry. Phytomedicine 2014;21:1534-42.
5. Shi X, Li X, He J, Han Y, Li S, Zou M, *et al.* Study on the antibacterial activity of *Bergenia purpurascens* extract. Afr J Tradit Complement Altern Med 2014;11:464-8.
6. Rajbhandari M, Mentel R, Jha PK, Chaudhary RP, Bhattarai S, Gewali MB, *et al.* Antiviral

activity of some plants used in Nepalese traditional medicine. Evid Based Complement Alternat Med 2009;6:517-22.

7. Spiridonov NA, Konovalov DA, Arkhipov VV. Cytotoxicity of some Russian ethnomedicinal plants and plant compounds. Phytother Res 2005;19:428-32.
8. Bhandari MR, Nilubon JA, Hung G, Kawabata J. Glucosidase and amylase inhibitory activities of Nepalese medicinal herb *Pakhanbhed* (*Bergenia ciliata* Haw). Food Chem 2007;106:247-52.
9. Verma P, Gauttam V, Kalia AN. comparative pharmacognosy of *Pashanbhed*. J Ayurveda Integr Med 2014;5:104-8.
10. de Oliveira CM, Nonato FR, de Lima FO, Couto RD, David JP, David JM, *et al*. Antinociceptive properties of *Bergenin*. J Nat Prod 2011;74:2062-8.
11. Golovchenko VV, Bushneva OA, Ovodova RG, Shashkov AS, Chizhov AS, Ovodov Iu, *et al*. Structural study of bergenan, a pectin from *Bergenia crassifolia*. Bioorg Khim 2007;33:54-63.
12. Churin AA, Masnaia NV, Sherstoboev EY, Suslov NI. Effect of *Bergenia crassifolia* extract on specific immune response parameters under extremal conditions. Eksp Klin Farmakol 2005;68:51-4.
13. Cheel HJ. Biological Activities and Chemical Content of *Glycyrrhiza* species. Dissertation Thesis 2010. Faculty of Pharmacy, Hradec Králové, Charles University; 2010.
14. Cheel J, Antwerpen PV, Tůmová L, Onofre G, Vokurková D, Zouaoui-Boudjeltia K, Vanhaeverbeek M, Nève J. Free radical-scavenging, antioxidant and immunostimulating effects of a licorice infusion (*Glycyrrhiza glabra* L.). Food Chem 2010;122:508-17.
15. Popov SV, Popova GY, Nikolaeva SY, Golovchenko VV, Ovodova RG. Immunostimulating activity of pectic polysaccharide from *Bergenia crassifolia* (L.) fritsch. Phytother Res 2005;19:1052-6.
16. Nazir N, Koul S, Qurishi MA, Taneja SC, Ahmad SF, Bani S, *et al*. Immunomodulatory effect of *Bergenin* and *Norbergenin* against adjuvant-induced arthritis – A flow cytometric study. J Ethnopharmacol 2007;112:401-5.