

## The effects of chloroform, ethyl acetate and methanolic extracts of *Brassica rapa* L. on cell-mediated immune response in mice

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### Abstract

Turnips with a long history of usage, are helpful in preventing breast and prostate cancer, inflammation and body's immune system dysfunction. In this study, we investigated the effects of chloroform, ethyl acetate and methanolic extracts of *Brassica rapa* L. on cell-mediated immune response in mice. Chloroform, ethyl acetate and methanolic extracts of *B. rapa* glands were prepared by maceration method. To study the effects of *B. rapa* on acquired immunity, groups of Balb/c mice (n=8) were used. Sheep red blood cell (SRBC) was injected (s.c.,  $1 \times 10^8$  cells/ml, 0.02 ml) and 5 days later, different extracts (10, 100 and 500 mg/kg), betamethasone (4 mg/kg) and Levamisol (4 mg/kg) as a positive control and normal saline as a negative control were given i.p. After 1 h SRBC was injected to footpad (s.c.,  $1 \times 10^8$  cells/ml, 0.02 ml) and footpad swelling was measured up to 72 h. To investigate the effects of *B. rapa* on innate immunity the same procedure was used, but animals only received one injection of SRBC 1 h after i.p. injection of test compounds. Our findings showed that SRBC induced an increase in paw swelling with maximum response at 6-8 and 2-4 h for innate and acquired immunity, respectively. Betamethasone inhibited and levamisol increased paw thickness in both models. In both innate and acquired immunity models, chloroform, ethyl acetate and methanolic extracts of *B. rapa* glands significantly and dose-dependently reduced paw thickness. Ethyl acetate extract showed better effect. As glucosinolates are better extracted by ethyl acetate, it may be concluded that they are contributed in the more pronounced effects of ethyl acetate extract.

**Keywords:** *Brassica rapa*; Turnip; Acquired immunity; Innate immunity

### INTRODUCTION

It has been shown that the use of herbals and botanicals are getting more popular in all parts of the world. National Center for Complementary and Alternative Medicine of the United State reported about 19% of adult Americans are using some form of natural products (1). It should be considered that in the developing countries about 80% of population continues the use of traditional medicine in primary medical problems indicating high use of herbal remedies in these countries (2). The use of some of the herbals in different product forms that are available in the market are based on their traditional applications, and there is lack of scientific investigation about their effects on health (3). During the last decades, we are facing with a

new generation of botanical therapeutics including plant-derived pharmaceuticals and multicomponent botanical drugs (4). Recently scientists are becoming more interested in finding new therapeutic agents with immunomodulatory activities (5).

Substances, modifying the activities of the immune system are referred to as immunomodulators. There are two categories of immunomodulators; immunostimulators which stimulate immune system and immunosuppressors which inhibit host parameters that are normal or already activated (6). It has been shown that one of the major problem in cancer therapy when cytotoxic agents are used, is their undesired immunosuppression effects, such as bone marrow suppression, resulting in cytopenia and subsequent suppression of humoral, non-

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specific and specific cellular responses (5-7). Therefore, some agents with ability to alleviate these side effects are needed.

The use of immunomodulators with plant origin has been getting more attention in the field of cancer research. It has been shown that some plants such as *Tinospora cordifolia* (8), *Allium hirtifolium* Boiss (9), *Allium fistulosum* L (10), *Withania somnifera* (11) and *Piper longum* (12) have immunomodulatory activities. Also, some studies have shown that some components such as polysaccharides, lecithin (13), glucosinolates (14), proteins and peptides (15) which present highly in plants modulate the immune system (6).

Previous studies had shown that some plants from *Brassicaceae* possess immunomodulatory activity (16). Also, they are rich sources of glucosinolates and isothiocyanate (17).

Turnip belongs to the genus *Brassica* and the family *Brassicaceae*. The family *Brassicaceae* comprises many important vegetable crops including: *Brassica* (turnip, broccoli, Brussels sprouts, cabbage, mustard, rutabaga.), *Lepidium* (cress), *Nasturtium* (watercress), *Raphanus* (radish), and *Crambe* (oil-seed) (18,19). Vegetables from *Brassicaceae* family, have received widespread notice by scientists as examples of medicinally significant foods (20). Traditionally, white cabbage leaves were used in the treatment of many diseases where problems due to immune system are involved including rheumatoid, diabetes mellitus, gastric ulcer, cirrhosis and cancer. The immunomodulatory effects of white cabbage leave relates to its polysaccharides and hydrosoluble compounds.(16). In this study we sought to determine if different extracts of *B. rapa* L. have any effect on cellular immunity in mice.

## MATERIALS AND METHODS

### Plant material

The glands of *B. rapa* were purchased from a local market of Isfahan, Iran. The plant specimen was identified by Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

### Preparation of extracts

Air-dried and powdered glands of the plant

(100 g) were percolated with 400 ml of methanol, chloroform and ethyl acetate for 48 h. After filtration, the extracts were evaporated until dryness using a vacuum evaporator (21,22).

### Animals

Six-to eight-weeks old Balb/c male mice were purchased from Pasteur Institute (Tehran, Iran). Treatment of the animals was in accordance with institutional guidelines. They were maintained in a temperature and light-controlled environment with free access to standard rodent chow and water.

### Sheep red blood cell-induced paw thickness

To investigate the effects of *B. rapa* extracts on acquired immunity, twelve groups of 8 Balb/c mice receiving methanolic, chloroform and ethylacetate extracts (10, 100 and 500 mg/kg), betamethasone (4 mg/kg) and Levamisol (4 mg/kg) as positive controls and normal saline as a negative control were used. Sheep red blood cells (SRBC) were obtained from Shahrekord slaughterhouse and prepared by centrifugation followed by 3 times washing with normal saline. SRBC was injected subcutaneously (s.c) on the shaved back of animals ( $1 \times 10^8$  cells/ml, 0.02 ml) on the zero. The mice were challenged on day 5 by injecting  $10^8$  SRBC (20  $\mu$ l, s.c) into the right hind footpad. Footpad thickness was measured with an engineer's caliper up to 72 h after antigen challenge, and degree of footpad swelling was calculated as below:

$$\text{Percent increase} = \frac{(\text{footpad thickness after antigen challenge} - \text{footpad thickness before antigen challenge})}{\text{footpad thickness before antigen challenge}} \times 100.$$

To investigate the effects of *B. rapa* extracts on innate immunity, animals received one injection of SRBC on the zero day into the footpad (s.c) 1 h after i.p. injection of test compounds (23,24).

### Statistical analysis

SIGMASTAT™ (Jandel Software, San Raphael, CA) was used to perform statistical analyses. The data are presented as means  $\pm$  S.E.M. The significance of differences between various experimental groups were determined by analysis of variance (ANOVA), followed by Student-Newman-Keuls post hoc test. Significance was assumed at 5% level.

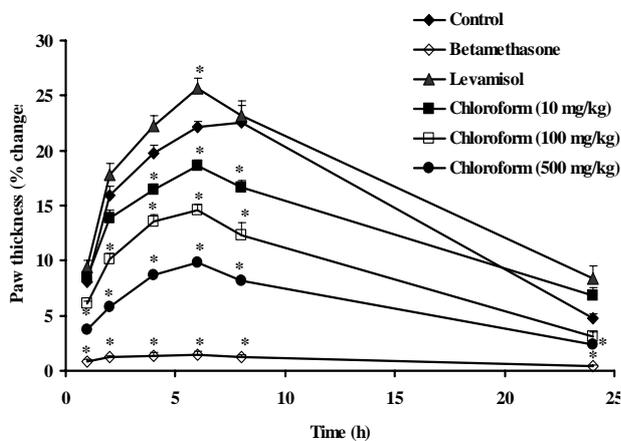
## RESULTS

### Extraction yield

Using percolation method for extraction, the extract yield of semi-solid masses after evaporation and solvent removal of chloroform, ethyl acetate and methanolic extracts of *B. rapa* were 25, 10 and 30%, respectively.

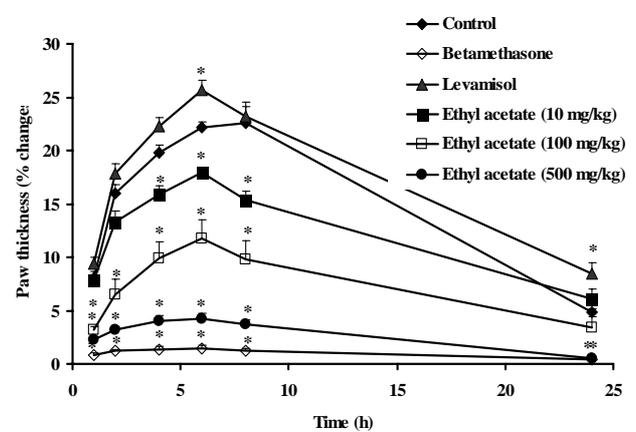
### Effects of chloroform, ethyl acetate and methanolic extracts of *B. rapa* on innate immunity

The animals were randomly divided into

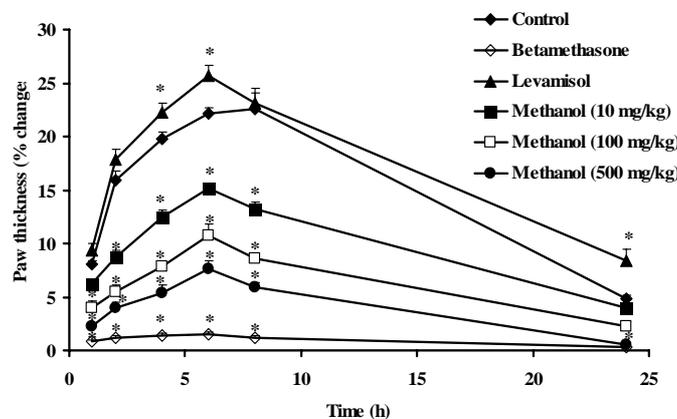


**Fig. 1.** Effect of chloroform extract of *B. rapa* on the innate immunity. Animals received 20  $\mu$ l SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (10, 100 and 500 mg/kg, i.p.) one h before the antigen challenge. Paw thickness was measured up to 48 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  SEM. Groups of 8 mice per condition was used. \* =  $P < 0.05$  compared with the control group.

twelve groups, each composed of eight mice. Nine groups of animals received a single dose of chloroform, ethyl acetate or methanolic extract of *B. rapa* (10-500 mg/kg) while the remainder groups (negative and positive controls) received normal saline, betamethasone (4 mg/kg) or levamisol (4 mg/kg), respectively. After 1 h, SRBC was injected into footpad and footpad thickness was measured up to 24 h. SRBC injection significantly increased paw thickness with maximum response occurring at 6-8 h ( $P < 0.05$ , Figs. 1-3). Betamethasone

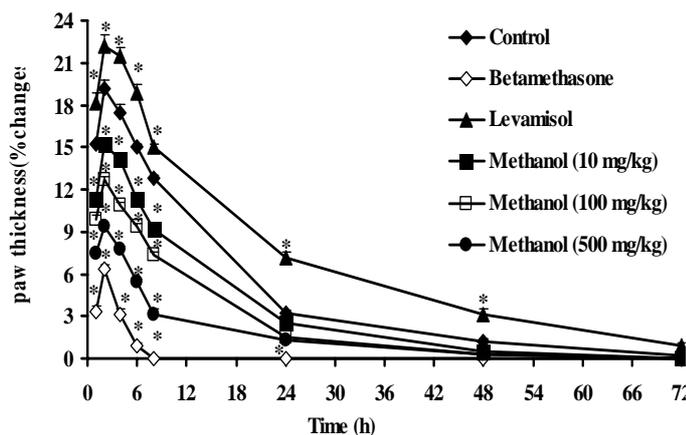


**Fig. 2.** Effect of ethyl acetate extract of *B. rapa* on the innate immunity. Animals received 20  $\mu$ l SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (10, 100 and 500 mg/kg, i.p.) one h before the antigen challenge. Paw thickness was measured up to 48 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  SEM. Groups of 8 mice per condition was used. \* =  $P < 0.05$  compared with the control group.

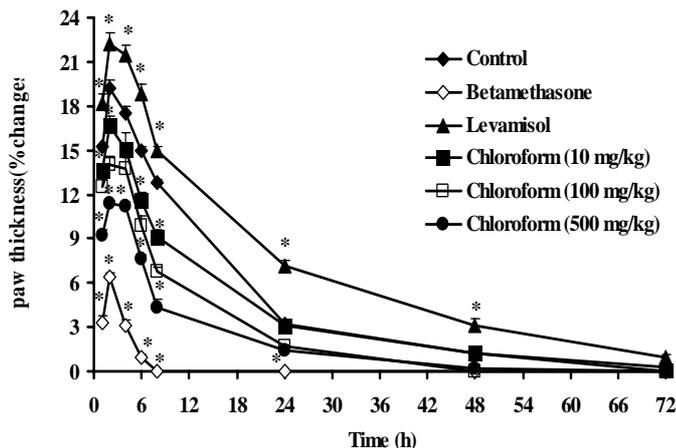


**Fig. 3.** Effect of methanolic extract of *B. rapa* on the innate immunity.

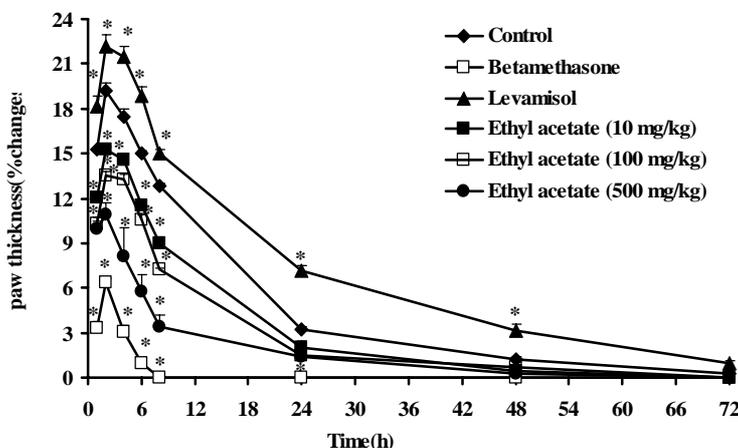
Animals received 20  $\mu$ l SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (10, 100 and 500 mg/kg, i.p.) one h before the antigen challenge. Paw thickness was measured up to 48 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  SEM. Groups of 8 mice per condition was used. \* =  $P < 0.05$  compared with the control group.



**Fig. 4.** Effect of methanolic extract of *B. rapa* on the acquired immunity. Animals received 20  $\mu$ l SRBC to their shaved back. After 5 days, a hypersensitivity response was elicited by injecting SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (10, 100 and 500 mg/kg, i.p.) one h before the antigen challenge. Paw thickness was measured up to 72 h after the antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  SEM. Groups of 8 mice per condition was used. \* =  $P < 0.05$  compared with control group.



**Fig. 5.** Effect of chloroform extract of *B. rapa* on the acquired immunity. Animals received 20  $\mu$ l SRBC to the shaved back. After 5 days, a hypersensitivity response was elicited by injecting SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (10, 100 and 500 mg/kg, i.p.) one h before the antigen challenge. Paw thickness was measured up to 72 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  SEM. Groups of 8 mice per condition was used. \* =  $P < 0.05$  compared with control group.



**Fig. 6.** Effect of ethyl acetate extract of *B. rapa* on the acquired immunity. Animals received 20  $\mu$ l SRBC to the shaved back. After 5 days, a hypersensitivity response was elicited by injecting SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (10, 100 and 500 mg/kg, i.p.) one h before the antigen challenge. Paw thickness was measured up to 72 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  SEM. Groups of 8 mice per condition was used. \* =  $P < 0.05$  compared with control group.

administration (4 mg/kg) significantly inhibited the paw thickness while Levamisol (4 mg/kg) significantly increased this parameter (Figs. 1-3). Chloroform, ethyl acetate and methanolic extracts of *B. rapa* gland significantly and dose-dependently reduced paw thickness ( $P < 0.05$ , Figs. 1-3).

**Effects of chloroform, ethyl acetate and methanolic extracts of *B. rapa* on acquired immunity**

Different groups of mice (similar to the innate immunity model) were used. They were challenged with SRBC on day zero and on day 5 one h after receiving i.p. injection of

betamethasone, paw thickness was measured up to 72 h. SRBC injection significantly increased paw thickness with maximum response at 2-4 h ( $P<0.05$ ) (Figs. 4-6). Percent of changes in paw thickness in control group was significantly higher than that of the innate immunity ( $P<0.05$ ). Administration of betamethasone (4 mg/kg) significantly decreased paw thickness and levamisol (4 mg/kg) significantly increased paw thickness (Figs. 4-6). Our findings showed that, chloroform, ethyl acetate and methanolic extracts of *B. rapa* glands significantly and dose-dependently decreased paw thickness ( $P<0.05$ ).

## DISCUSSION

Nowadays our awareness regarding the role of vegetables in the human diet in prevention of the disease is increasing. One of the important areas of research is modulation of immune system. In the management and treatment of inflammation and allergic diseases the immune system need to be suppressed, while stimulation of the immune system is highly desirable for the treatment of HIV, immunodeficiency and infectious diseases (25). It has been demonstrated that some vegetables from the genus Brassica (brassicaceae family) including cabbage, Brussel's sprouts possess immunomodulatory properties (16,25). In the current study the effects of different extracts of *B. rapa* L., from the genus Brassica, was investigated in the animal models of delayed-type hypersensitivity (DTH).

DTH is a model of cell-mediated response that is well-defined *in vivo*. DTH reaction can be quantified by measuring the amount of paw thickness after injection of an antigen (23,24,26). Betamethasone (4 mg/kg), a well known immunosuppressive drug inhibited paw thickness and levamisol (4 mg/kg), an immunostimulating drug increased paw thickness in both the innate and acquired immunity models indicating the accuracy of the methods used in these experiments (Figs. 1-6).

Solvents with different polarities including methanol, chloroform and ethyl acetate were chosen to partially fractionize phytochemicals of *B. rapa* glands (27). Chloroform, ethyl

acetate and methanolic extracts of *B. rapa* significantly and dose dependently reduced paw thickness in both models ( $P<0.05$ ) (Figs. 1-6). Phytochemicals including saponins, tannins, terpenoids, flavons and polyphenols could be retrieved by extraction with methanol. Also chloroform extracts contain phytochemicals such as terpenoids and flavonoids (28). As the extent of the effects of chloroform and methannolic extracts of *B. rapa* were similar, it may be concluded that this response is attributed to the presence of terpenoids and polyphenols. Our findings showed that the ethyl acetate extract of *B. rapa* had greater effect than those of chloroform and methanolic extracts. It has been shown that ethyl acetate can extract tannins, flavonoids, glycosides, resins and glucoseinolates (29).

Several organosulfur compounds including isothiocyanates (ITCs) and dithiolethiones have been reported in cabbage, Brussel's sprouts and other cruciferous vegetables. They occur in cruciferous vegetables as thioglucoside conjugates called glucosinolates (6). They are very abundant in *brassicaceae* (17). Previous studies have shown high levels of glucosinolates in *B. rapa* (30). Although, glucosinolates are biologically inactive they are rapidly hydrolysed by myrosinase to yield glucose and instable aglycons and then breackdown to isothiocyanates (31). Isothiocyanates had free radical scavenging, antioxidant, anticancer, antiinflammatory and immunomodulatory properties (32-36).

## CONCLUSION

Based on these finding it seems reasonable to assume that glucosinolates contributed in the more pronounced effects of ethyl acetate extract. However further pharmacological and phytochemical studies are needed to identify the constituents of *B. rapa* and precisely evaluate their immunomodulating activities and mechanisms.

## ACKNOWLEDGMENT

This study was supported by a grant from the Research Council of Isfahan University of Medical Sciences, Isfahan, Iran.

## REFERENCES

- Patricia M, Barnes MA, Powell-Griner E, McFann K, Nahin RL. Complementary and alternative medicine use among adults: United States. *Sem Integrative Med.* 2004;2:54-71.
- Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: a review. *J Ethnopharmacol.* 2004;93:123-132.
- Scoglione F. Molecular bases of immunomodulatory activity of medicinal extracts. *Revista de Fitoterapia.* 2006;6:43-46.
- Raskina I, Ribnickya DM, Komarnytskya S, Ilicb N, Poulevb A, Borisjuk N. *et al.* Plants and human health in the twenty-first century. *Trends Biotechnol.* 2002;20:522-531.
- Geetha S, Singh V, Ram MS, Ilavazhagan G, Banerjee PK, Sawhney RC. Immunomodulatory effects of Seabuckthorn (*Hippophaea rhamnoides* L.) against chromium (VI) induced immunosuppression. *Mol Cell Biochem.* 2005;278:101-109.
- Thejass P, Kuttan G. Immunomodulatory activity of Sulforaphane, a naturally occurring isothiocyanate from broccoli (*Brassica oleracea*). *Phytomedicine.* 2007;14:538-545.
- Yan N, Chen ZJ. Intrinsic antiviral immunity. *Nature Immunol.* 2012;13:214-222.
- Sonel M, Kuttan G. Immunomodulatory and antitumour activities of *Tinospora cordifolia*. *Fitoterapia* 1999;70:35-43.
- Jafarian A, Ghannadi A, Elyasi A. The effects of *Allium hirtifolium* Boiss. on cell-mediated immune response in mice. *Iranian J Pharm Res.* 2003;2:51-56.
- Jafarian A, Sajjadi SE, Mohammadzadeh A. The effects of methanolic and chloroformic extracts of *Allium fistulosum* L. on cell-mediated immune response in mice. *Res Pharm Sci.* 2007;1:59-65.
- Davis L, Kuttan G. Immunomodulatory activity of *Withania somnifera*. *J Ethnopharmacol.* 2000;71:193-202.
- Sunila ES, Kuttan G. Immunomodulatory and antitumour activity of Piper longum Linn. and Piperine. *J Ethnopharmacol.* 2004;90:339-346.
- Hajto T, Hostanska K, Gabius HJ. Modulatory potency of the B-galactoside specific lecithin from mistletoe extract (Iscador) on the host defense system *in vivo* in rabbits and patients. *J Cancer Res.* 1989;49:4803-4808.
- Manesh C, Kuttan G. Effect of naturally occurring isothiocyanates on the immune system. *Immunopharmacol Immunotoxicol.* 2003;25:451-459.
- Kuttan G, Kuttan, R. Immunomodulatory activity of a peptide isolated from *Viscum album* extract. *Immunol Invest.* 1992;21:285-296.
- Miron A, Hancianu M, Aprotosoiaie AC, Stanescu GO. Contributions to chemical study of the raw polysaccharide isolated from the fresh pressed juice of white cabbage leaves. *Rev Med Chir Soc Med Nat Iasi.* 2006;110:1020-1026.
- Schreiner M, Schonhof I, Krumbein A. Effect of nitrogen sulfur supply on yield and glucosinolates content of turnip root. *Tsingwa Uni Press.* 2005;47:358-359.
- Østergaard L, King GJ. Standardized gene nomenclature for the Brassica genus. *Plant Meth.* 2008;4:10
- Cartea MA, Francisco M, Soengas P, Velasco P. Phenolic compounds in Brassica vegetables. *Molecules* 2011;16:251-280.
- Beecher CWW. Cancer preventive properties of varieties of *Brassica oleracea*: a review. *Am J Clin Nutr.* 1994;59:1166S-1170S.
14. "Osterreichisches Arzneibuch". Wien: Editio Nona Osterreichisches-taatsdruckerei;1960.
- Momeni T. Herbal Extracts. Tehran: Reza Press; 2000.
- Malik F, Singh J, Khajuria A, Suri KA, Satti NK, Singh S, *et al.* A standardized root extract of *Withania somnifera* and its major constituent Withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. *Life Sci.* 2007;80:1525-1538
- Belkaid Y, Valenzuela JG, Kamhawi S, Rowton E, Sacks DL, Ribeiro JM. Delayed-type hypersensitivity to *Phlebotomous papatasi* sand fly bite: an adaptive response induced by the fly. *Proc Natl Acad Sci.* 2000;97:6704-6709.
- Koko WS, Mesaik MA, Yousaf S, Galal M, Choudhary MI. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. *J Ethnopharmacol.* 2008;118:26-34.
- Barrington-Leigh J. The *in vitro* induction of delayed-type hypersensitivity to allo-antigens of the mouse. *J Immunol Meth.* 1984;69:149-163.
- Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas, 2nd ed. Springer, Munchen.1996.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A review. *Int Pharm Sciencia.* 2011;1:98-106.
- Adeshina1 GO, Onalapo1 JA, Ehinmidu1 JO, Odama LE. Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja. *Nigeria J Med Plants Res.* 2010;4:649-658.
- Barbieni G, Pernic R, Maggio A. Glucosinolates profile of *Brassica rapa* L. subsp. *Sylvestris* L. *J Food Chem.* 2008;107:1687-1691.
- Cartea ME, Francisco M, SEongas P, Velasco P. Phenolic compounds in Brassica vegetables. *Open access molecules.* 2011;16:251-280.
- Tawfiq N, Heaney RK, Plumb JA. Dietary glucosinolates as blocking agents against carcinogenesis-Breakdown products assessed by induction quinine reductase activity in murine hep cell. *Carcinogenesis.* 1995;16:1191-1196.
- Carlson DG, Daxenbichler ME, Tookey HL. Glucosinolate in turnip top and roots. *J Am Soc Holticultural Sci.* 1997;112:179-183.

34. Tannin-Spitz T, Bergman M, Grossman S. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. *Biochem Biophys Res.* 2007;364:181-186.
35. Bouie PJ, Clarak A, Lamprecht J, Freestone M, Pool EJ, Liebenberg RW. *et al.* The effects of B-sitosterol (BSS) and B-sitosterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: inhibition of post marathon immune suppression and inflammation. *Int J Sports Med.* 1999;20:258-262.
36. Preobrazhenskaya MN, Bukhman VM, Korolev AM. Ascorbigen and other indole-derived compounds from *Brassica rapa* vegetables and their analogs as anticarcinogenic and immunomodulating agents. *Russian Acad Med Sci.* 2002;14:181-187.