www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(5): 810-814

www.thepharmajournal.com
Received: 19-03-2019
Accepted: 06-04-2019

Ashwani Kumar
Research Scholar, JJT University, Jhunjhunu, Rajasthan, India

Dr. Vikas Anand
Sardar Bhagwan Singh University, Balawala, Dehradun, Uttarakhand, India

Corresponding Author:
Ashwani Kumar
Research Scholar, JJT University, Jhunjhunu, Rajasthan, India

Screening of immunomodulatory potential of stem bark extracts of *bauhinia variegata LINN.*

Ashwani Kumar and Dr. Vikas Anand

Abstract
Presently, around the world, there is an expansion in ailments particularly irresistible infections that requires proficient body guard instruments to control them through the cycle of immunomodulation. In Africa and Asia, about 80% of the populace have been accounted for to rely upon conventional medication for their essential medical care needs including immunomodulation.
The immunomodulatory study was performed by using aqueous, methanolic, chloroform and petroleum ether as a solvent. For this study, various immunomodulatory evaluations methods like neutrophil adhesion test, haemagglutination titre test and phagocytic index carbon clearance assay were performed. In this study various immunomodulatory test of stem bark extracts of Bauhinia Variegata Linn. were performed to screened out the immunomodulatory potential.
From the result, it was clearly indicated from tables and figures that all the stem bark extracts of B. Variegata Linn. was show immunomodulatory potency when compared with placebo or control group.

Keywords: immunomodulatory activity, Bauhinia variegata Linn, Stem bark

1. Introduction
Currently, worldwide, there is an increase in diseases especially infectious diseases that requires efficient body defence mechanisms to control them through the process of immunomodulation. Various allopathic drugs or medicines are used to modulate the immune system. However, these drugs are very expensive for poor people, they are not easily accessible, and in most cases they are associated with adverse drug reactions. As a result, the majority of people especially in the rural areas of the developing world turn to the use of alternative herbal medicines from medicinal plants that are widely accepted, accessible, cheaper, and assumed to have fewer side effects. In Africa and Asia, about 80% of the population have been reported to depend on traditional medicine for their primary health care needs including immunomodulation. A number of medicinal herbs have long been used and reported to boost the immune system or to modulate it and they are used putatively to treat and prevent various disease conditions worldwide.(Nishijima David L; Wisner, David H; Holmes, James F, 2016)

Method & Material
Chemicals: The chemicals used were of analytical grade. Aqueous, petroleum ether, chloroform, methanol were the chemicals used in the study.

Animals and their maintenance: Healthy adult albino rat of either sex of weight range 150-220 g, housed in the animal house of the ***Department of Pharmaceutical Sciences, Faculty of Medical Science & Health, Gurukula Kangri Vishwavidyalaya***, ***Haridwar*** were used. Animals were fed with standard diet. Animals were maintained under standard conditions of temperature (25◦C ± 5◦C) and relative humidity (55 ± 10%), and 12/12 hr light/ dark cycle. They were housed in standard polypropylene cages with wire mesh top husk bedding. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee and were in the accordance with the guidelines of the CPCSEA, Ministry of Forest and Environment, Government of India.

Extraction of the plant material: Plant materials were washed with water and shade dried. The dried bark was crushed to coarse powder by the grinder. The powdered material was defatted with petroleum ether (60-80 ºC) and then successively extracted in Soxhlet apparatus with aqueous, methanol, petroleum ether and chloroform as a solvent. Extract obtained was

passed through the Whatman filter paper No.1 and the solvent was evaporated with the help of a distillation unit and the spongy mass so obtained was dried in a desiccator. The extract was concentrated for further studies on water bath at 40 ºC (Kumar *et al*., 2016) [2].

Neutrophil Adhesion

The rats were treated orally with vehicle, extracts and standard drugs (Livamesole & Azathioprine) for 14 days. On day 14, blood samples were collected from the retro- orbital plexus into heparinised vials and analysed for differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibres for 10 min at 37 °C. The incubated blood samples were again analysed for DLC. The percentage of neutrophils in the treated and untreated blood was determined and the difference was taken as index of neutrophil adhesion

Calculated as follows,

$$\text{Neutrophil adhesion} = \frac{\text{NIU - NIT}}{\text{NIU}} \times 100$$

Where, NIU: Neutrophil Index before hatching with nylon fiber.

NIT: Neutrophil Index after hatching with nylon fiber

Haemagglutination test

Rats were pre-treated with the drugs and extracts for 14 days and each rat was immunized with 0. 5 × 109 sheep red blood cells (SRBCs) intraperitoneal, including control rats. The day of immunization was referred to as day 0. The drug treatment was continued for 14 more days and blood samples were collected from each rat at the end of the drug treatment and the titre value was determined by titrating serum dilutions with SRBC (0. 025 × 109 cells) in micro titre plates. The plates were incubated at room temperature for 2 hr and examined visually for agglutination. The minimum volume of serum showing haemagglutination was expressed as hemagglutination (HA) titre.

Phagocytosis Using Carbon Clearance Method

Animals were treated with the drug or vehicle orally for 10 days. After 48 hr of the last dose of the drug, animals were injected 0.1 ml of carbon suspension via the tail vein. Blood samples were withdrawn at 0 and 15 min after injection. A 50 µl blood sample was mixed with 4 ml of 0. 1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm.

The phagocytic index K was calculated using the following equation:

$$K = (\text{Log OD1-Log OD2})/15$$

Where OD 1 and OD 2 are the optical densities at 0 and 15 min respectively.

Result

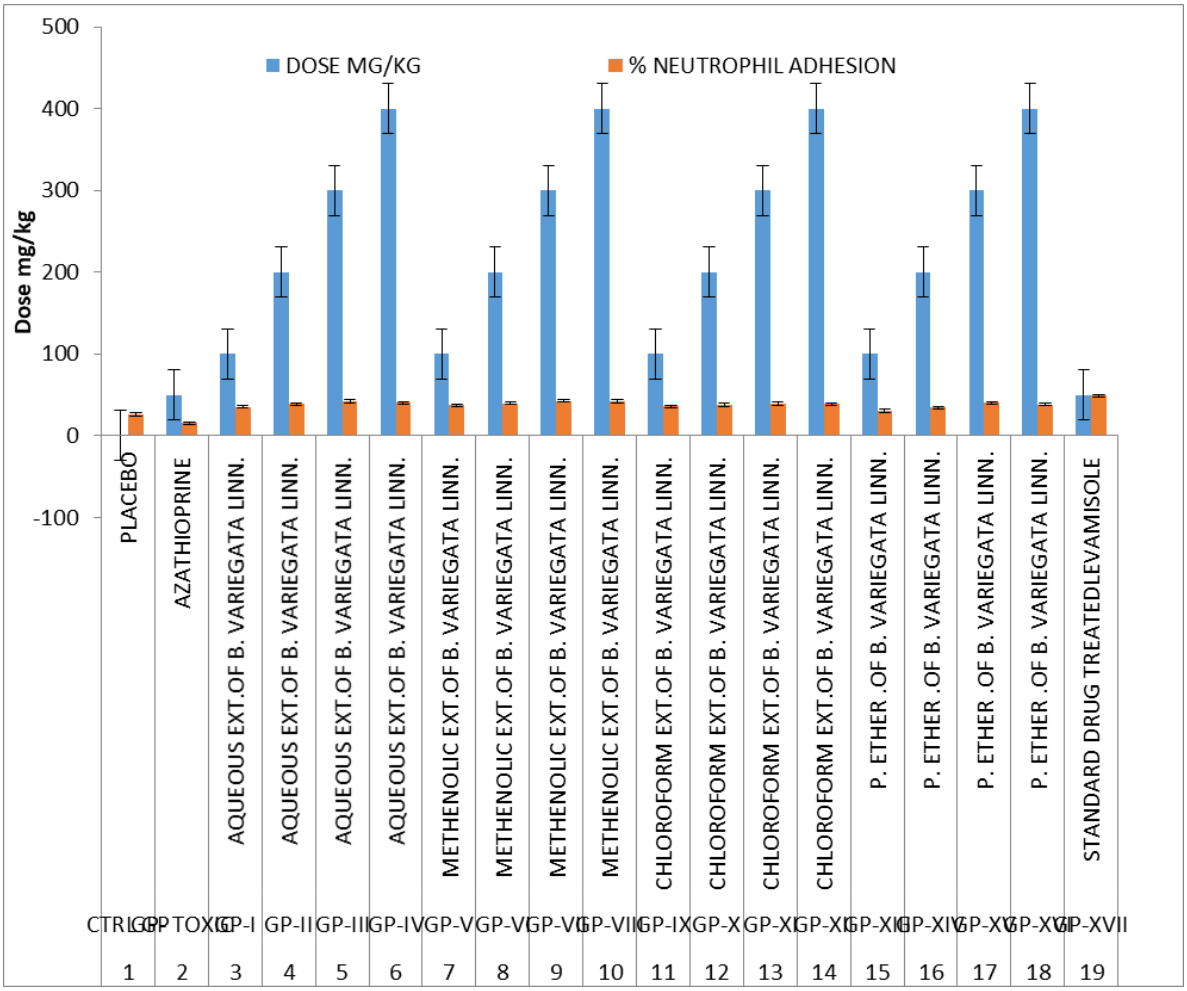

Fig 1: Neutrophil adhesion test

Table 1: Neutrophil Adhesion test

S.N	Group	Treated with	DOSE MG/KG	% Neutrophil adhesion
1	CTRL GP	PLACEBO	-	25.82
2	GP- TOXIC	AZATHIOPRINE	50	15.54
3	GP-I	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	100	35.25
4	GP-II	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	200	38.65
5	GP-III	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	300	42.05
6	GP-IV	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	400	40.23
7	GP-V	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	100	36.95
8	GP-VI	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	200	39.65
9	GP-VII	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	300	42.35
10	GP-VIII	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	400	42.06
11	GP-IX	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	100	35.89
12	GP-X	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	200	37.58
13	GP-XI	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	300	39.27
14	GP-XII	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	400	38.87
15	GP-XIII	P. ETHER. EXT. OF ***B. VARIEGATA LINN.***	100	30.21
16	GP-XIV	P. ETHER. EXT. OF ***B. VARIEGATA LINN.***	200	34.25
17	GP-XV	P. ETHER. EXT. OF ***B. VARIEGATA LINN.***	300	40.29
18	GP-XVI	P. ETHER. EXT. OF ***B. VARIEGATA LINN.***	400	38.33
19	GP-XVII	STANDARD DRUG TREATEDLEVAMISOLE	50	49.05

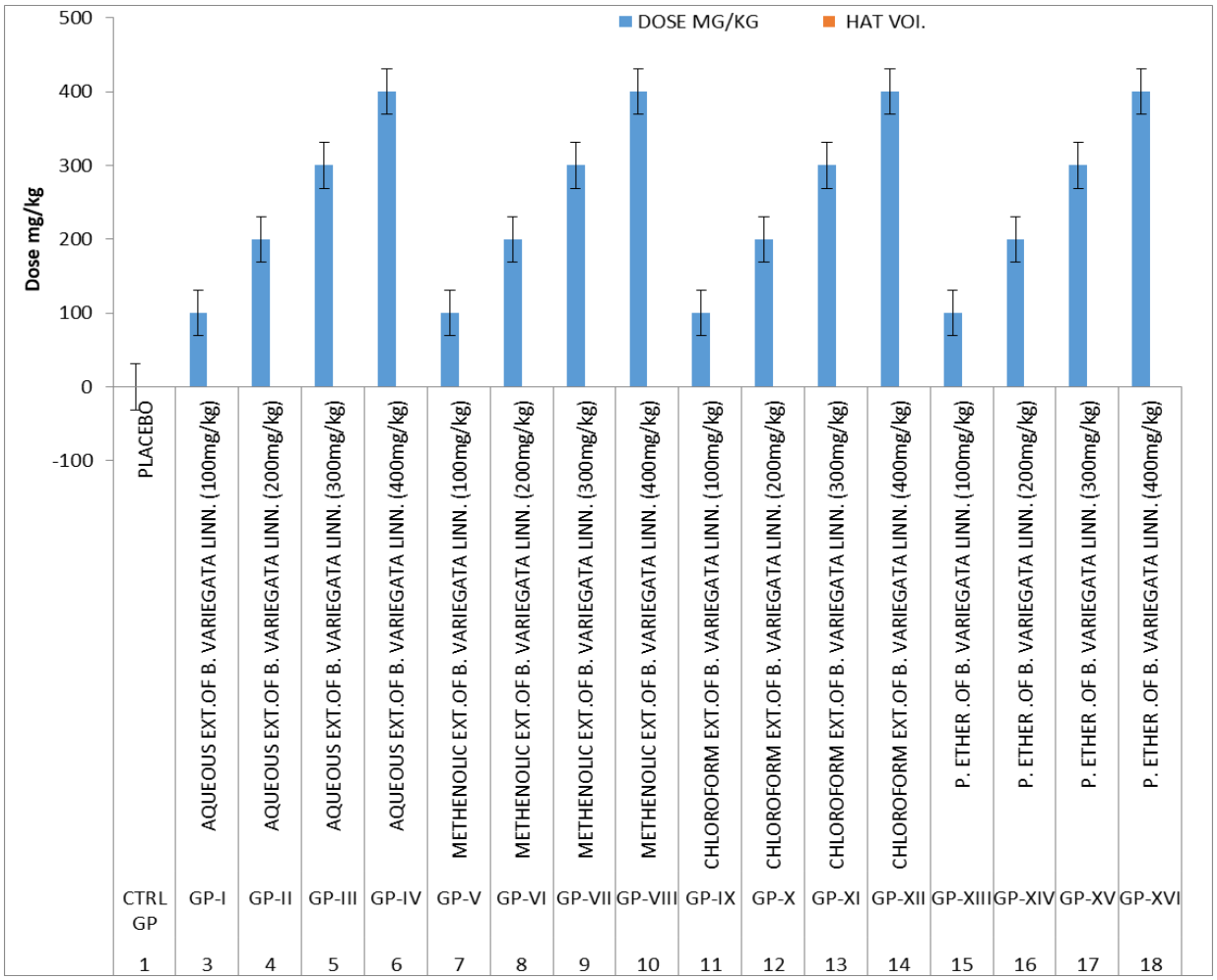

Fig 2: Haemagglutination test

Table 2: Haemagglutination test

S.N	GROUP	TREATED WITH	DOSE MG/KG	HAT VOI.
1	CTRL GP	PLACEBO	-	0.272
3	GP-I	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	100	0.194
4	GP-II	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	200	0.164
5	GP-III	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	300	0.093
6	GP-IV	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	400	0.112
7	GP-V	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	100	0.218

8	GP-VI	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	200	0.173
9	GP-VII	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	300	0.112
10	GP-VIII	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	400	0.163
11	GP-IX	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	100	0.111
12	GP-X	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	200	0.098
13	GP-XI	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	300	0.076
14	GP-XII	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	400	0.396
15	GP-XIII	P. ETHER. EXT OF***B. VARIEGATA LINN.***	100	0.135
16	GP-XIV	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	200	0.11
17	GP-XV	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	300	0.041
18	GP-XVI	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	400	0.121

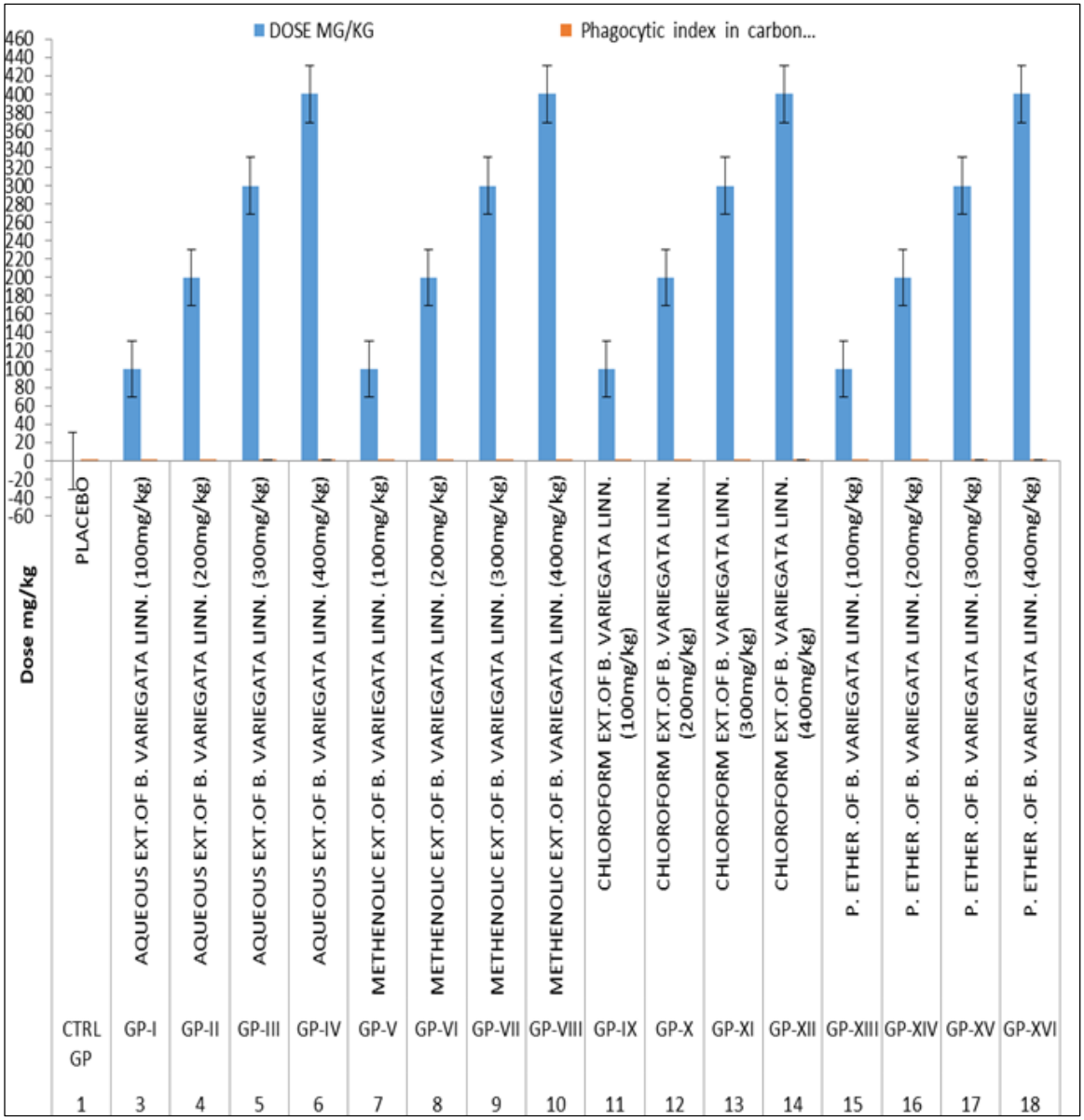

Fig 3: Phagocytic index (Carbon Clearance)

Table 3: Phagocytic index (Carbon Clearance)

S.N	Group	Treated with (Dose mg/kg)	Dose MG/KG	Phagocytic index in carbon clearance assay
1	CTRL GP	PLACEBO	-	0.081
3	GP-I	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	100	0.194
4	GP-II	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	200	0.307
5	GP-III	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	300	0.42
6	GP-IV	AQUEOUS EXT.OF ***B. VARIEGATA LINN.***	400	0.533
7	GP-V	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	100	0.218
8	GP-VI	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	200	0.273
9	GP-VII	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	300	0.328
10	GP-VIII	METHENOLIC EXT.OF ***B. VARIEGATA LINN.***	400	0.383

11	GP-IX	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	100	0.211
12	GP-X	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	200	0.298
13	GP-XI	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	300	0.385
14	GP-XII	CHLOROFORM EXT.OF ***B. VARIEGATA LINN.***	400	0.472
15	GP-XIII	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	100	0.135
16	GP-XIV	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	200	0.289
17	GP-XV	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	300	0.443
18	GP-XVI	P. ETHER. EXT OF ***B. VARIEGATA LINN.***	400	0.597

Conclusion

Based on the findings from the study, all the stem bark extracts of B. Variegata Linn. show significant neutrophil adhesion percentage when compared with placebo and standard drug Livamesole responses in rats. In hemagglutination test, petroleum ether extract shows the most significant HAT volume as compared to other B. Variegata stem bark extract. But all the extracts show significant values as compared to control or placebo group. In case carbon clearance test all the stem bark extracts of B. Variegata Linn. show significant phagocytic index as compared to placebo group. This could be attributed to the different macronutrients, micronutrients and phytochemicals present in the plant and hence the reason for its use in local communities to alleviate various disease conditions.

Acknowledgement

The authors are sincerely thankful to the *Department of Pharmaceutical Sciences, Faculty of Medical Science & Health, Gurukula Kangri Vishwavidyalaya, Haridwar* for providing Laboratory facilities.

Reference

1. Fahey JW. Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1. TFL J. 2005; 1:1-15.
2. Kumar A, Anand V, Dubey RC, Goel K. Toxicological evaluation of Bauhinia variegata Linn. for estimating the neurological, hematological and physical alterations in the albino mice. Environment Conservation Journal, 17(3):97-102. https://doi.org/10.36953/ECJ.2016.17318
3. Nishijima David L, Wisner, David H, Holmes, James F, DKS. 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*. 2016; 176(6):139-48. ttps://doi.org/10.1016/j.physbeh.2017.03.040
4. Janeway CA, Travers P, Jr Walport M, Shlomchik MJ. The immune system in health and disease: immunobiology. 5. New York: Garland Publishing, 2001, 1-312.
5. Sudha P, Asaq SB, Dhamingi SS, Chandrakala GK. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in animals. Indian J Pharmacol. 2010; 54:133-40.
6. Banji OJ, Banji D, Kavitha R. Immunomodulatory effects of alcoholic and hydroalcoholic extracts of Moringa oleifera Lam leaves. Indian J Exp. Biol. 2012; 50:270-6. [PubMed: 22611915]
7. Bart HJ, Pilz S. Industrial scale natural products extraction. 1. Wiley-VCH Verlag & Co., KGaA; Boschstr Weinheim, Germany. 2011; 12:69469. 56Available at: http://site.iugaza.edu.ps/tbashiti/files/2013/02/2.Industrial_Scale_Natural_Products_Extraction.pdf
8. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with many medicinal uses. Phytother Res. 2007; 21:17-25. [PubMed: 17089328]
9. Fuglie LJ. The miracle tree: Moringa oleifera: natural nutrition for the tropics. Dakar, Senegal: Church World Service. 1999; 1:63.