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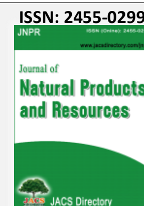

Evaluation of Immunomodulatory Activities of Metabolic Extract of *Dalbergia sissoo* Bark

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ABSTRACT

Development in clinical and experimental immunology strongly suggests that many infectious diseases and disorders arise because of stressful environmental conditions associated with suppression of immune system. It is evident that certain types of stress evoke physiological changes that influence susceptibility to infection and malignance. Currently there has been an increased interest globally to identify immunostimulant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and food industry. The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. The Present Study aimed to investigate Immunomodulatory effect of *Dalbergia sissoo* bark by using four methods named as Humoral immune response, WBC count, cellular immune response, and Carbon clearance test. Administration of *Dalbergia sissoo* produced a significant stimulation of immune system. And also it can be concluded that the immunostimulatory property of extract was dose dependent.

1. Introduction

Immunity is the reaction of cells and tissues to foreign substances or pathogens. The immune response requires the timely interplay of multiple cell types within specific microenvironments to maintain immune homeostasis. Innate immunity is the rapid response to the first encounter to a pathogen. A consequence of an initial exposure is adaptive or acquired immunity. The contributors to innate immunity are neutrophils, monocytes and macrophages. Lymphocytes (B and T) as well as cytokines are directly involved in generation of adaptive immunity shown in Fig. 1 [1, 2].

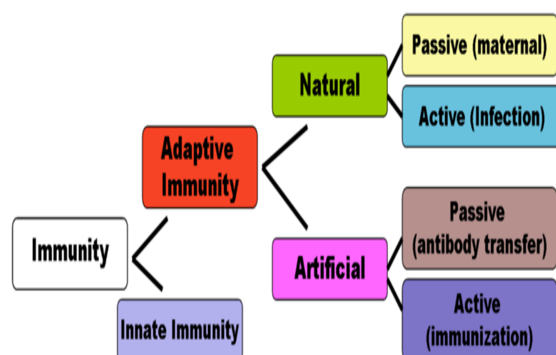

Fig. 1 Components of immune system

An immunomodulator may be defined as a substance, biological or synthetic which can stimulate, suppress or modulate any of the components of immune system. Most drugs however do not have effects on only one receptor, so an immunomodulator may be at the same time an immunosuppressant and an immunostimulant, on different targets within the immune system. Products that are not single chemical entities, such as herbal extracts and impure products, may have even greater plurality of effect. Many species of plants, depending on the specific extraction conditions used, have immunomodulatory effects. Many synthetic immunomodulatory compounds have shown side effects, which have stimulated the interest to search natural immunomodulatory agents [3-17]. Moreover they also used as nutraceuticals and phytoceuticals, as they have significant role on the status of human disease prevention [12, 17]. The study was aimed at investigating the effect of Immunomodulatory property of Dalbergia *sissoo* bark.

2. Experimental Methods

2.1 Collection of Plant Material

Dalbergia sissoo is a large genus of small to medium size tree. Found in local areas of Tirupati (Chittoor), A.P. The bark was botanically authenticated by Dr. K. Madhava chetty. Collected bark was dried and size reduced into powder with the help of laboratory mixer and sieved.

2.2 Successive Solvent Extraction (Soxhlation)

Soxhlation is a process of continuous extraction in which the same solvent can be circulated through the extractor several times. The process involves extraction followed by evaporation of the solvent. The vapours of the solvent are taken to a condenser and the condensed liquid will be returned to the drug for continuous extraction. Soxhlet apparatus consist of a body of extractor attached with a side tube and siphon tube.

The powdered bark was packed in the soxhlet apparatus directly or in a thimble pack. The vapor pass through the side tube and the condensed liquid gradually increases the level of liquid in the extractor and in the siphon tube. A siphon is set up when the liquid reaches the point of return and the contents of the extraction chamber are transferred to the flask. The cycle of solvent evaporation and siphoning back can be continued many times without changing the solvent for efficient extraction.

In the present study the extracts were prepared by 200 g of finely powdered bark was extracted with different solvents in their order of increasing polarity *viz.*, petroleum ether (60-80 °C), chloroform, ethyl acetate, methanol and water in soxhlet apparatus for 8hrs. The extracts were collected evaporated under reduced pressure at low temperature (30 °C), until soft mass obtained and dried in a desiccator.

2.3 Acute Toxicity Studies

The toxicity study aim was to establish the therapeutic index i.e., the ratio between the pharmacologically effective dose and the lethal dose [ED_{50}/LD_{50}] on the same strain and species of animal. The greater the therapeutic index, the safer the drug. This study was performed with Male Swiss Mice. The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (acute

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toxic class method). The acute toxic class is a step wise procedure with three animals of a single sex in each step.

2.2.1 Animals

Male Swiss Albino Mice, Aged 4 weeks (body weight: 20±5 g) were used for the present study.

2.3.2 Plant Materials

Metabolic extract of *Dalbergia sissoo* bark was used in the present study.

2.3.4 Method

Three mice weighing between 25-30 gm were used for study. The starting dose level of methanolic extract of *Dalbergia sissoo* bark was 200 mg/kg b. w, p.o. Body weight of the mice before and after treatment were noted and any changes in skin and fur, eyes and mucous membranes and also autonomic, central nervous systems, behavior pattern were observed and also signs of tremors, convulsions, salivation, diarrohea, lethargy sleep and coma were noted. The onset of toxicity and signs of toxicity was also to be noted. The mice were then observed for another 24 hrs. No mortality and signs of behavioral changes were observed with 200 mg/kg b.w., then after one week a single dose of 500 mg/kg was administered and observed for toxicity for 24 hr. Then after one week followed dose of 1000 mg/kg and observed.

The Metabolic extract of *Dalbergia sissoo* bark was found to be safe since no animal died even at the single dose of 1000 mg/kg when administered orally, and the animals did not show any gross behavioral changes. Hence, 1000 mg/kg considered as the safe dose. Thus the LD_{50} value of the extract was >1000 mg/kg.

2.3.5 Immunomodularoty Property of Metabolic Extract of Dalbergia Sissoo Bark

2.3.5.1 Animals

Male Wistar Albino rats (150-200 g) and Swiss Albino Mice (25-30 g) were purchased from Mahaveera Enterprises, Hyderabad. The animals were acclimatized for about 7 days prior to dosing. Cage numbers and individual markings were made for identification. The animals were housed three per cage of same sex in polypropylene cages (38 cm X 23 cm X 10 cm) with a bedding of paddy. Pellet chow feed standard diet, under good management conditions and water *ad libitum* was provided to the animals. Temperature of 20-25 °C and 12 hours each of dark and light cycle was maintained. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional Animal Ethical Committee, Vaagdevi College of pharmacy, Warangal vide approval number 2011/10/3/15.

2.3.5.2 Test Extracts

The DSME dose of 250 and 500 mg/kg body weight was used. Control saline (0.9% w/v NaCl) was used as a general vehicle.

2.3.5.3 Standard

Levamisole 50 mg/kg body weight was used as standard.

2.3.5.4 Route of Administration of Test Compounds and Standard

The test and standard drugs were prepared in accurate dose and administered orally by an oral feeding needle inserted into the pharynx.

2.3.6 Treatment Schedule for Assesing Immunomodulatory Property of Methanolic Extract of Dalbergia sissoo Bark

Experimental rats were randomly divided into four groups and each group consists six animals (n=6) which is shown in Table 1.

Table 1 Grouping of animals for HA titer, WBC count and cellular immune response

S. No	Group	Treatment	Purpose
I	Control	Normal saline	To observe control Immunological parameters
II	Standard	Levamisole 50 mg/kg p.o./7 days	Comparison of test drug with standard
III	Test 1	DSME 250 mg/kg-p.o/7 days	To evaluate the efficacy of low dose of test drug
IV	Test 2	DSME 500 mg/kg-p.o/7 days	To evaluate the efficacy of high dose of test drug

2.3.7 Humoral Immune Response (Antibody (Ha) Titer)

Injecting rats i.p. with SRBCs suspended in control saline sensitizes them for elicitation of DTH and also induces antibody formation. This system has major advantage i.e., it enables two components of immune response to be measured in the same species under ideal conditions and is relatively simple and inexpensive to perform.

2.3.7.1 Requirements

- Antigen (sheep red blood cells)
- Normal saline
- Alsever's solution
- Microtitre plate (96 well plates)
- Plethysmometer
- 1% SRBCs suspension control saline

2.3.7.2 Alsever's Solution

Alsever's solution is an isotonic balanced salt solution consisting of glucose, 2.05%; sodium chloride, 0.42%; tri–sodium citrate, 0.8% and citric acid, 0.55%. It is routinely used as an anticoagulant / blood preservative, and permits the storage of whole blood for approximately 2 weeks in a refrigerator at 2-8 °C.

2.3.7.3 Antigenic Material

The sheep red blood cells (SRBCs) were used as an antigenic material. The sheep blood was obtained from slaughter house collected in Alsever's solution. During the experimentation, adequate amount of SRBCs were washed 3 times with pyrogen free control saline (0.9% w/v Nacl). The settled SRBCs were found to be 4.8 × 106 cells/mm^3 (by haemocytometer) and used for immunization and challenge.

Animals from all the groups were kept fasting over night before the day of starting the experiment. The animals were immunized by injecting 50 µL of SRBCs suspension containing 4.8 × 106 cells/ mm^3 intraperitonilly on day 0. Drugs of different concentrations i.e., 250, 500 mg/kg were administered to the respective groups orally for 7 days. Blood samples were collected in micro centrifuge tubes from individual animal by retro orbital puncture on day 8. The blood samples were centrifuged and serum was obtained.

Antibody levels were determined by the haemagglutination technique. Briefly equal volumes of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25 µL volume of control saline, in U-bottomed micro titration plates were added 25 µL of freshly prepared 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37 °C for 2 hrs and examined visually for agglutination as shown in Fig. 2. The highest dilution of test serum causing visible haemagglutination was taken as the antibody titer [3, 4].

2.3.8 White Blood Cell (Wbc) Count Principle

The known volume of blood is taken and diluting to a more no. of times using WBC diluting fluid and changing then to a chamber whose dimensions are known. The dilution is in 20 (or) 10 and the volume of chamber 1/160 mm^3.

2.3.8.1 Procedure

A drop of blood sample was withdrawn from the retro orbital venous plexus of rats. The collected blood was sucked up to 0.5 mark of WBC diluting pipette. The tip was cleaned and diluting fluids is sucked up to mark 11. The fluids were thoroughly mixed by rotating the pipette between the palms of two hands. A clean cover slip was placed over counting chambers. First few drops of diluting fluid present in capillary tube were discarded. A tiny drop was collected at the tip of pipette and was touched at junction of cover slip at the slide. Fluid was drawn in to chambers by capillary action. There should not be any air bubble. Examined under 10 x. Count the five fields. Finally Calculate the WBCs/ mm3 by adding the cells in the 5 groups and multiplying by 40 [5].

2.3.9 Cellular Immune Response

After blood collection on day eight the thickness (mL) of the right hind foot pad was measured using plethysmometer. The rats were then challenged by injection of 25 µL of 4.8 × 106 cells/mm^3 SRBCs subcutaneously into right hind foot pad. Foot thickness was measured again after 24 hrs after this challenge. The difference between the pre and post challenge foot thickness was taken as a measure of DTH [3, 4].

2.4 Carbon Clearance Test

Experimental mice were randomly divided into four groups and each group consists of six animals (n=6) which is shown in Table 2.

Table 2 Treatment schedule for carbon clearance test

S.No.	Group	Treatment	Purpose
I	Control	Normal saline	To observe control Immunological parameters
II	Standard	Levamisole 50 mg/kg p.o./7 days	Comparison of test drug with standard
III	Test 1	DSME 250 mg/kg-p.o/7 days	To evaluate the efficacy of low dose of test drug
IV	Test 2	DSME 500 mg/kg-p.o/7 days	To evaluate the efficacy of high dose of test drug

Phagocytosis is a process by which certain body cells, collectively known as phagocytes, ingest and removes microorganisms, effector malignant cells, inorganic particles and tissue debris (87). The selected plant extracts were subjected to carbon clearance test by the following procedure.

Adult male Swiss mice divided into four groups consisting of six animals each. The mice were deprived of food for 24hours with free access to water. After 24 hours, Levamisole (50 mg/kg), and methanolic extract of 250 mg/kg and 500 mg/kg dose was selected for screening and they were administered orally for 7 days.

On day 8, the mice were injected with 0.1 mL of carbon ink (Camel fountainpen ink) suspension (1.6% v/v in 1% Gelatin, in saline) via the tail vein. Blood samples (about 50 µL) were drawn (in 0.15% w/v disodium EDTA solution, 50 µL) from the retro orbital vein, immediately (0 min) and 15 minutes after injection. A 25 µL sample was mixed with 0.1% sodium carbonate solution (2 mL) and the absorbance was measured at 660 nm taking 0.1% sodium carbonate solution as blank [4, 5].

The carbon clearance was calculated using the following equation:

$$\text{Carbon clearance} = \frac{\text{LogOD}_1\text{-LogOD}_2}{T_2\text{-}T_1}$$

Where, OD1, OD2 are the optical densities at T1 and T2 respectively.

T_1 ---- 0 min T_2 ---- 15 min

2.5 Statistical Analysis

All the data was expressed as Mean ± S.D. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dennett's test using computer based fitting program (Prism graph pad version 5.0). Statistical significance was set accordingly.

3. Results and Discussion

3.1 Immunomodulatory Activity

Immunomodulatory property of *Dalbergia sissoo* methanolic extract was screened by using four methods named as Humoral immune response, WBC count, cellular immune response, and carbon clearance test.

In humoral immune response, the methanolic extract showed the haemagglutination titre (dilution) at 128 times for 250 mg/kg and 256 times for 500 mg/kg b.w. and standard that is Levamisole at 512 times which were comparable with the Control group value that is 2 times. The effect of DSME on humoral immune response was depicted in Table 3 and Fig. 3.

In WBC count, the methanolic extract increased significantly (p<0.001) the WBC count to 5933 cells/cmm at 250 mg/kg and 9100 cells/cmm at 500 mg/kg dose and 10016 cells/cmm in standard group (p<0.001) which was 4068 cells/cmm in Control group animals. The effect of DSME on WBC count was depicted in Table 3 and Fig. 4.

Table 3 Effect of methanolic extract of *Dalbergia sissoo* bark (DMSE) on antibody titer, WBC count, cellular immune response and phagocytic response

S.no	Group	HA titer (Dilution)	WBC count (X 1000/mm^3)	DTH Response (Paw edema)Ml	Phagocytic Response
1.	Control	2 times	4.06±0.057	0.170±0.011	0.022±0.004
2.	DSME (250 mg/kg)	128 times	5.93±0.15***	0.213±0.010*	0.045±0.004***
3.	DSME (500 mg/kg)	256 times	9.10±0.1***	0.304±0.014***	0.052±0.001***
4.	Levamisole (50 mg/kg)	512 times	10.01±0.015***	0.390±0.01***	0.059±0.001***

All values are shown as Mean ± SD and n=6.
*P < 0.05 – Statistically significant;
**P < 0.01 – Statistically very significant;
***P < 0.001 – Statistically very highly significant in response to control

In cellular immune response, the extract showed a marked increase in the paw volume which was monitored by plethysmometer. It showed 0.213 mL and 0.304 mL increase in paw volume at dose levels of 250 mg (p<0.001) and 500 mg/kg b.w. (p<0.001) respectively which was comparable with the Control group 0.17 mL and standard group which showed 0.39 mL (p<0.001). The effect of DSME on cellular immune response was depicted in Table 3 and Fig. 5.

In carbon clearance test, extract showed a recognizable phagocytic index of 0.045 and 0.052 at dose levels of 250 mg and 500 mg/kg b.w. (p<0.001), respectively and standard group showed at 0.059 (p<0.001), which were comparable with the control group value that is 0.022 The effect of DSME on carbon clearance test was depicted in Table 3 and Fig. 6.

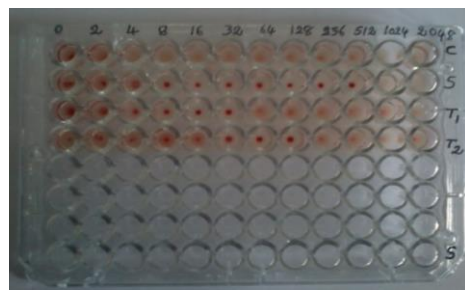

Fig. 2 Microtitre plate for Humoral immune response

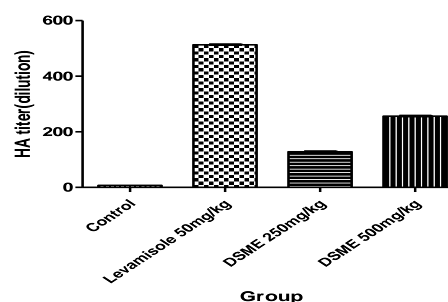

Fig. 3 Effect of DSME on Humoral immune response

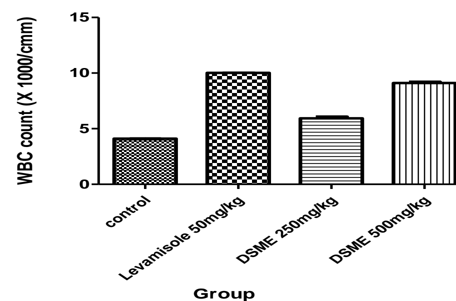

Fig. 4 Effect of DSME on WBC count

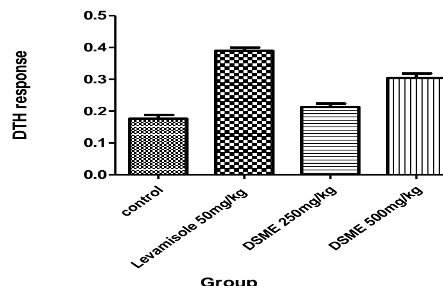

Fig. 5 Effect of DSME on cellular immune response

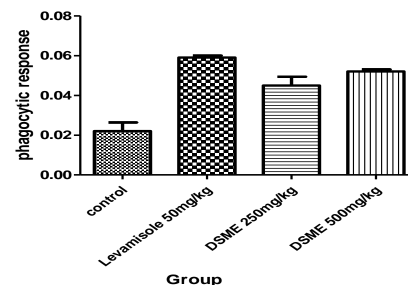

Fig. 6 Effect of DSME on Phagocytic response

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Acute oral toxicity study was carried out to determine the safe dose of *Dalbergia sissoo bark*. The procedure was carried according to the OECD guidelines. The results of the study revealed that, the safe dose of *Dalbergia sissoo* was 1000 mg/kg b.w. and the extract selected for screening of immunomodulatory activity at a dose of 250 and 500 mg/kg b.w.

Pharmacological evaluation of immunomodulatory property was performed by using Humoral immune response, WBC count, cellular immune response, and Carbon clearance test.

In Humoral immune response, the extract showed the haemagglutination titre (dilution) at 128 times for 250 mg/kg and 256 times for 500 mg/kg b.w and standard that is levamisole at 512 times which was Control group value that is 2 times.

In WBC count, the extract increased significantly ($p<0.001$) the WBC count to 5933 cells/cmm at 250 mg/kg and 9100 cells/cmm at 500 mg/kg dose and 10016 cells/cmm in standard group ($p<0.001$) which was 4068 cells/cmm in Control group animals. This shows that the extract exerting potent immunostimulant property.

In cellular immune response, the extract showed a marked increase in the paw volume which was monitored by plethysmometer. It showed 0.216 mL and 0.304 mL increase in paw volume at dose levels of 250 mg ($p<0.01$) and 500 mg/kg b.w. ($p<0.001$) respectively which was comparable with the Control group 0.17 mL and standard group which showed 0.39 mL ($p<0.001$). This result of delayed type hypersensitivity indicates that the extract was capable of stimulating the body immune system so that the host defence mechanism will be activated.

In carbon clearance test, extract showed a recognisable Phagocytic index of 0.045 and 0.052 at dose levels of 250 mg and 500 mg/kg b.w.($p<0.001$), respectively and standard group showed at 0.059 ($p<0.001$), which were comparable with the Control group value that is 0.022. Phagocytosis is a process by which certain body cells, collectively known as phagocytes, ingest and removes microorganisms, effector malignant cells, inorganic particles and tissue debris. As extract showing the good carbon clearance, the above results are indicating that the extract shows good immunostimulant property.

4. Conclusion

Herbal formulation may be therefore recommended for use as positive immunomodulator. There are several botanical products with potential therapeutic applications because of their high efficacy, low cost and low toxicity. Based on the above observations it can be concluded that the extract possess potent immunostimulant activities. Administration of *Dalbergia sissoo* produced a significant stimulation of immune system. And also it can be concluded that the immunostimulatory property of extract was dose dependent.

References

[1] M.N. Levy, B.M. Koeppen, B.A. Stanton, Principles of physiology, Philadelphia, Mosby, Elsevier, 2006, pp.417-428.

[2] N.S. Balekar, D.K. Jain, Screening methods for immunomodulatory agents - a review, Ind. Drugs 43 (2006) 525-534.

[3] A. Puri, R.P. Saxena, K.C. Saxena, Immunostimulant agents from *Andrographis paniculata*, J. Nat. Prod. 56 (1993) 995-999.

[4] S. Dash, L.K. Nath, S. Bhise, P. Kar, S. Battacharya, Stimulation of immune function activity by the alcoholic root extract of *Heraclem napalence,* Ind. J. Pharm. 38(5) (2006) 336-348.

[5] B.V. Ghule, P.D. Murugananthan, P.G. Nakaht, Yeolao, Immunostimulant effects of *Capparis zeylaica Linn* leaves, J. Ethnopharm. 108 (2006) 311-315.

[6] R.H. Gokani, S.K. Lahiri, D.D. Santani, M.B. Shah, Evaluation of immunomodulatory activity of *Clerodendrum phlomidis* and *Premna integrifolia*, Int. J. Pharm. 3(4) (2007) 352-356.

[7] S. Hemanth, K.M.D. Yaseen, Immunomodulatory plants: a phytopharmacological review, Pharmacog. Rev. 1(2) (2007) 248-260.

[8] Y.S. Jae, Y.S. Ji, S.Y. Yeon, O.Y. Hyun, K.R. Dong, Immunostimulating effect of acidic polysaccharides extract of *Panex ginseng* on macrophage function, Immunopharm. Immunotox. 24(3) (2002) 469-482.

[9] A.D. James, Godwin, Judiducellier, A.K. Duke, Hand book of medicinal herbs, 2nd Ed., USA, 2006, pp. 133.

[10] P. Phatru, M.D. Basheeruddin, Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals, Saudi Pharm. J. 18 (2010) 161-165.

[11] N.S. Balekar, D.K. Jain, Screening methods for immunomodulatory agents - a review, Ind. drugs 43 (2006) 525-534.

[12] K.H. Benny, T.J. Vanitha, Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review, Current Med. Chem. 11 (2004) 1423-1430.

[13] P. Bhushan, G. Manish, Botanical immune drugs: scope and opportunities, Drug Discov. Today 10 (2005) 1-8.

[14] J.R. Caroline, V. Rama, K.G. Ramesh, Immunomodulatory activity of aqueous extract of *Ocimum sanctum* in rat, Int. J. Pharm. Biomed. Res. 2(1) (2011) 33-38.

[15] A.S. Damre, A.S. Gokhale, K.R. Phadle, K.R. Kulkarni, M.N. Saraf, Studies on the immunomodulatory activity of flavonoidal fraction of *Tephrosia purpurea*, Fitoterapia 74 (2003) 257-261.

[16] S.Y. Gabhe, P.A. Tatke, T.A. Khan, Evaluation of the immunomodulatory activity of methanol extracts of *Ficus benghalensis* in rats, Ind. J. Pharm. 38(4) (2006) 271-275.

[17] A.M. Mujumdar, A.V. Misar, A.S. Upadhye, Antidiarrhoel activity of ethanol extract of the bark of *Dalbergia lanceolaria*, J. Ethnopharm. 102 (2005) 213-216.