Int. J. Adv. Res. Sci. Technol. Volume 3, Issue 2, 2014, pp.119-125 # ICV 5.14

IJARST

International Journal of Advanced Research in Science and Technology

journal homepage: www.ijarst.com

ISSN 2319 – 1783 (Print)

ISSN 2320 – 1126 (Online)

Modulation of Mitogen-Response and Phagocytic Activities of Human Lymphocytes and Macrophages by Aqueous, Hexane and Methanol Extracts of Stem Bark of *Boswellia Dalzeilii*, Hutch (Family: *Burseraceae*)

OUMAR Mahamat[1,3*], TUME Christopher[2] and KAMANYI Albert[3]

[1]Department of Biological Sciences, Faculty of Science, University of Bamenda, Cameroon

[2]Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon

[3]Department of Animal physiology, Faculty of Science, University of Dschang, Cameroon

**** Corresponding authors: oumar.mahamat@yahoo.fr***

ARTICLEINFO

Article history:

Received 17 Sep. 2014

Accepted 10 Oct. 2014

Available online 12 Oct. 2014

Keywords:

Macrophage;
lymphocyte;
iNOS;
phagocytosis;
myeloperoxydase;
Boswellia dalzeilii
.

ABSTRACT

The barks of *Boswellia dalzeilii* (*B. dalzeilii*) have been traditionally used in Cameroon and other Africa countries. The aqueous extract (Aq-extract), methanol extract (Me-extract) and hexane extract (He-extract) of *Boswellia dalzeilii barks* were evaluated for immune modulatory activity using *in vitro* and *in vivo* methodologies. Effect of the extracts was evaluated at various concentrations (2560 – 160 µg/ml) for reduction of Nitro blue tetrazolium (NBT), inducible nitric oxide synthase (iNOS) and myeloperoxidase activity of macrophages. Particularly, aq-extract was evaluated in vivo for carbon clearance. The Aq-extract that evaluated for *in vivo* phagocytic activity by carbon clearance assay in mice, showed significant increase in the phagocytic index at 0.75, 1.25 and 2.5µg/kg dose. The extract showed stimulation reduction of NBT, nitric oxide production after blocking of iNOS using N^G-nitro-L-arginine methyl ester (L-NAME), and myeloperoxidase activity. The effect of the extract on lymphocyte proliferation and IL-2 production was evaluated. *B.dalzeilii* showed a significant effect on lymphocyte proliferation and IL-2 production. The results suggest that *B. dalzeilii* stimulated non-specific murine immune system, both *in vitro* and *in vivo*.

Introduction:

The immune system is known to be involved in the etiology as well as pathophysiologic mechanism of various diseases. This problem can be overcome by boosting the immune system by the use of immunomodulatory drugs of natural or synthetic origin. Only a few chemotherapeutic agents as immune potentiators are available today and most of them are cytotoxic and exert a variety of side effects[1]. This has given rise to stimulation in the search for investigating natural resources showing immune modulatory activity. Immunotherapy using plants can provide an alternative to conventional chemotherapy for a variety of diseases. Several types of immune modulators have been identified, including substances isolated and purified from plants[2]. It has become a global concern to treat and manage the course of the debilitated diseases such as cancer, HIV infections and mycobacterial tuberculosis/leprosyetc where patients among others, suffer drastically from a state of immune dysfunction often associated with suppression of immune cells and molecules. Immunology is probably one of the most rapidly developing areas of biomedical research and has great promises with regard to prevention and treatment of wide range of disorders[3].

Commonly consumed raw materials of plant origin are the most important sources of bioactive compounds. The importance of several plants in maintenance of health and in protection from chronic diseases is of growing interest among scientists, food manufacturers, consumers and health organizations[4]. Many *Boswellia* plant used in the traditional medical system have been shown to possess immune depressive activity acting at different levels of the immune system[5]. *B. dalzeilii* is a medicinal plant claimed to possess number of therapeutic uses including immunomodulatory.

Bdalzeilii been used as a medicinal plant for a long time in Africa. Barks are used in a traditional herbal remedy for various diseases such as AIDS, inflammation, diarrhea. *Boswellia* plant is used for conditions involving inflammation and infectious diseases; however the immunostimulatory potential of *B. dalzeilii* plant on immune system has not yet been explored. In preliminary studies, we found that it had effect on monocytes/macrophages activities[6]. Therefore, the objective of the present study was to broaden the knowledge on immunomodulatory activity of stem barks of this plant.

2. Materials and Methods:

2.1. Reagents:

All chemicals and reagents used in the current study were of analytical grade and mostly purchased from Sigmachemicals (Germany).

2.2. Collection of plant material:

Barks of trunks of *B. dalzeilii* were collected from the surrounding of Garoua, Cameroon. Plant was identified and authentified in National herbarium (4359/SRFK). The collected barks were carefully shade dried and ground to course powder.

2.3. Preparation of extracts:

2.3.1. Preparation of hexane and methanolic extract:

Dried and powdered barks were placed in the beakers and sufficient quantity of hexane was firstly added so as to submerge the plant material. The material was kept for 72h. Each day, the material was stirred mechanically for one hour and then double filtered through the filters. Finally the material was filtered through No 3 wathman paper and the filter. The residue from hexane extraction was used for extraction of methanolic following similarly. The extracts were evaporated to dryness under reduced pressure on a rotavapour. Final drying was done in a vacuum desiccator.

2.3.3. Preparation of aqueous extract:

The dried and powdered plant material was extracted by boiling the powder in distilled water and filtered as above. The crude aqueous extracts were dried in a vacuum desicator. All the prepared extracts were weighted and stored at 4 C.

2.4. Immunomodulatory potential of different solvent extracts prepared from trunk bark of *B. dalzeilii:*

2.4.1. Lymphocyte isolation from blood:

Ten millilitres of blood (10 ml) were mixed aseptically in a sterile glass tube with a drop of preservative-free heparin (500 IU Leo) and allowed to stand in an incubator for 2hrs at 37°C. After sedimentation of the red blood cells, the plasma rich in white blood cells was used for mononuclear cell purification. The cells were separated and isolated by density-gradient centrifugation on Ficoll-hypaque[7]. After washing twice with PBS, the suspension containing a mixture of lymphocytes and monocytes was obtained.The cell number was counted with a haemocytometer by the trypan blue dye exclusion technique. Cell viability exceeded 95%.

2.4.2. Monocytes and *generation of Macrophages:*

Monocytes were separated from lymphocytes by adhesion on plastic support (flacon)[8]. Briefly, the suspension of mononuclear cells was cultured at a density of 8 x 10^6 cells/ml in 25 ml plastic culture bottles culture medium (MEM) supplemented with 5% foetal calf serum (FCS) and penicillin / streptomycin 100 U/ml. 12 hrs of incubation non-adhering cells were discarded and adhering cells that constitute monocytes were collected by adding EDTA solution at 2%. The monocytes were then concentrated by centrifugation at 1800 rpm for 10 minutes at room temperature. Monocytes at a density of $5x10^{10}$cells/ml were cultured in the presence of granulocyte macrophage colony-stimulating factor (40ng/ml) in culture medium (MEM) containing 5% foetal calf serum (FCS) and penicillin / streptomycin 100 U/ml at 37°C. On day 3, 90% of the medium was replaced with fresh medium and cytokine. Macrophages were then harvested on day 7 and washed for further assays.

2.4.3. Incubation of lymphocytes with plant extracts:

The sample extracts (dissolved in RMPI-1600) at different concentrations, were incubated with 2.5×10^6 cells lymphocyte**s/** well at 37 °C for 68hrs in humid saturated atmosphere.

2.4.4.T and B cell proliferation assay by MTT assay:

To evaluate the effect of different extracts prepared from *B. dalzeilii* on the proliferation of lymphocytes, the PBMNC suspension containing (2.5×10^5 cells/mL) were seeded in a 96-well culture plates (200µL/well) in triplicates. Cell proliferation was determined by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as previously described[9]. PBMNCs were treated with various concentrations of each extracts and sub-optimal concentrations of PHA (4µg/mL) and LPS (1µg/mL) were added to each well separately for priming T cells and B cells respectively. Plates were incubated at 37 °C for 68 h in a humid saturated atmosphere. After 72 h, 20 µL of MTT solution (5 mg/mL) was added to each well and the plates were incubated for 4 h. Thereafter, to each well, 100 µL of a working solution (192 µL DMSO with 8 µL 1 M HCl) was added and the absorbance was evaluated in an ELISA plate reader at 492 nm after 15 min.

2.4.5. Detection of lymphocyte mitogenic factor activity in culture supernatant fluid:

Lymphocyte mitigenic factor was detected in surpernatant collected from the culture activated by

PHA. The control supernatant were also collected and reconstituted with PHA and extract to give the same final concentration present in the stimulated cultures. Supernatant from sample were termed preincubated "P", the reconstituted "R" for control supernatant reconstituted and the control non-reconstituted were termed "C". The detection was based on stimulation of splenocytes that is indicative of IL-2 presence. Splenocytes were suspended at $1.5x10^6$ cells/ml in RPMI-1640 and distributed in 200 µl in microplate. Undiluted P, R and C supernatant fluids as wells as dilutions are added in 100µl volume to cells. After 24h, the proliferation was evaluated using MTT. The percentage corresponding to IL-2 effect was calculated as follow: % = $[(A_P-A_R) / A_C]$ x100 where A_P is the absorbance of culture in presence of P supernatant, A_R is the absorbance of culture in presence of R reconstituted supernatant and A_C absorbance of control culture[10].

2.4.6. Nitroblue Tetrazolium Reduction assay:

The NBT dye reduction assay was carried out as described previously[11]. Briefly, 50µl of 0.3% NBT solution in PBS (phosphate buffered saline, pH 7.4) was added to the 24h incubated cells (1×10^6 cells/ml) with *B. dalzeilii* extract, and the mixture was further incubated in CO_2 incubator. After incubation for 1h, the adherent macrophages were rinsed vigorously with complete RPMI medium, and washed four times with 200µl Methanol. After air-drying, formazan-deposits were solubilized in 120µl of 2M KOH and 140µl of DMSO. After homogenization of the contents of the wells, the OD was read at 630 nm by using a microplate reader. Stimulation index (SI) was calculated as the OD ratio of the treated and control macrophages[12].

2.4.7. Inducible Nitric Oxide Synthase iNOS activity

Inducible nitric oxide synthase activity of macrophages suspension was assessed spectrophotometerically by employing the method mentioned[13] using L-NAME. Briefly the macrophages were incubated with L-NAME, extracts and BCG at 37 °C for 24 hours in CO_2 chamber. The colour developed was measured spectrophotometrically at 540 nm against RPMI and Griess reagent as blank or NO_2 for standard curve. The results were expressed as mean± S.E.M. of concentration of nitrite production as NO produced.

2.4.8. Myeloperoxidase activity assay:

Myeloperoxidase activity was evaluated on isolated macrophages as per the earlier procedure[14]. Briefly, 24h incubated macrophages (5x105cells/ml) were washed three times with fresh complete RPMI medium. Then the mixture (100 µl) of *o*-phenylenediamine (0.4 g/ml) and 0.002% H2O2 in phosphate-citrate buffer (pH 5.0) was added to each well. The reaction was stopped after 10 min using 0.1 N H_2SO_4 and OD were measured at 490 nm. The myeloperoxidase stimulation index (SI) was calculated as the OD ratio of the treated and control cells.

2.4.9. *In vivo* phagocytic activity by carbon clearance assay:

Phagocytic activity of *B. dalzeilii* extract was determined as per the method described earlier[15]. Mice were divided into five groups, of six each. The control group received vehicle (H_2O distilled). Mice in the treatment groups were administered with orally MCM extract (0.75, 1.25and 2.5µg/kg) suspended in vehicle daily for 20 days. Colloidal carbon solution, Rotring ink® (Hamburg, Germany) was diluted with normal saline (1:8), and injected (0.01 ml/g body weight) was via tail vein to each mouse 24 h after last dose. Blood samples were drawn from retro-orbital plexus under ether anesthesia at 2 and 15 min after injection. Blood (25µl) was mixed with 0.1 % sodium carbonate (2 ml) for the lysis of erythrocytes OD was recorded at 660 nm. The phagocytic index (K) was calculated by using following equation: K= (ln OD1-ln OD2) / (T2-T1), where OD1 and OD2 are the optical densities at times T1 and T2, respectively[11].

2.5. Statistical analysis:

Data are expressed as Mean ± S.E.M. Statistical analysis of the data was performed on the original data by one-way analysis of variance ANOVA. Differences at $P < 0.05$ were considered to be statistically significant.

3. Results:

3.1. *In vivo* phagocytic activity by carbon clearance assay:

Macrophages accomplish nonspecific immune function through phagocytosis. *In vivo* phagocytic activity of Aq-extract of *B. dalzeilii* was determined by the carbon clearance assay in mice. The results of this assay are presented in Fig.1. The phagocytic index (K) for *B. dalzeilii* extract was significantly higher ($P<0.01$) at 0.75, 1.25 and 2.5 µg/kg compared to control group.

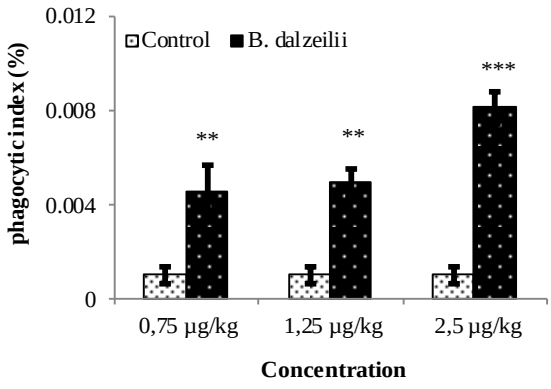

Fig. 1: Effects of aqueous extract of the stem bark of *B. dalzeilii* on phagocytic activity in mice. Each bar represents the mean ± SEM of 3 animals; $^{**}p<0.01$, $^{***}p<0.001$ statistically significant compared to control.

3.2. Effect of sample extracts on NBT reduction:

Results of NBT reduction potential revealed that incubation of lymphocytes with Me- and He-extracts of *B. dalzeilii* at concentrations (1280&2560 µg/ml) resulted in significant ($p<0.05$) rise in bioactivity as compared to positive. These two extracts, Me–extract and He-extract, showed maximum reduction potential while the He-extract produced a no significant reduction with maximum effect observed at concentration of 160 µg/ml and the minimum at 2560µg/ml (Table 1).

Table 1: In vitro effect of extract of *B. dalzeilii* on NBT reduction of isolated macrophages

Conc. (µg/ml)	Treatments		
	Aqueous Extract	Methanol Extract	Hexane Extract
Control	1,00±0,00	1,00±0,00	1,00±0,00
160	1,40±0,46	1,18±0,05	0,89±0,16
320	1,25±0,08	1,03±0,12	1,13±0,01
640	0,59±0,08	1,20±0,03	1,20±0,08
1280	0,63±0,04	1,53±0,04[a]	1,47±0,06[a]
2560	0,55±0,08	1,59±0,03[a]	1,88±0,14[a]
Positive	1,47±0,04[a]	1,47±0,04[a]	1,47±0,04[a]

The data is expressed as Mean ±S.E.M; n=3 wells. [a]*P<0.05 vs control.*

3.3. Effect of sample extracts on iNOS activity:

The study revealed that different extracts of *B. dalzeilii* at concentrations (160 to 100 µg/ml) significantly ($p<0.001$) enhanced the iNOS activity as compared to untreated control. But, only the sample of Aq-extract and Me-extract showed a significant iNOS activity at the concentrations of 640, 1280 and 2560 µg/ml (Fig.2).

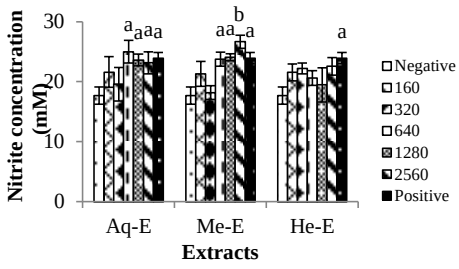

Fig. 2: Effects of extract of the stem bark of *B. dalzeilii* on iNOS activity LNAME-inhibited in macrophages. Each bar represents the mean ± SEM of 3 wells; [a]$p<0.05$ and [b]$p<0.01$ statistically significant compared to positive.

3.4. Effect of sample extracts on Myeloperoxidase activity assay:

The effect of *B. dalzeilii* extract on myeloperoxidase activity f macrophages is presented in Fig.3. The extract showed significant ($P<0.05$) stimulation of myeloperoxidase activity of macrophages at 640 - 2560µg/ml as compared to positive control, PHA.

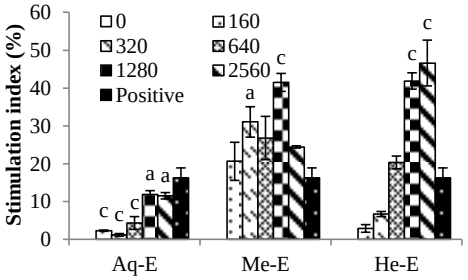

Fig. 3: Effects of extract of the stem bark of *B. dalzeilii* on myeloperoxydase activity in macrophages. Each bar represents the mean ± SEM of 3 wells; [a]$p<0.05$ and [c]$p<0.001$ statistically significant compared to positive.

3.5. Effect of sample extracts on lymphocyte proliferation induced by PHA:

The effect of various extracts prepared from barks stem of *B. dalzeilli* on PHA and LPS-stimulated lymphocyte proliferation was studied by MTT assay. Different concentration of extracts caused profound lymphocyte activation and triggered a significant ($p<0.05$) proliferation of lymphocytes which was observed in PHA stimulated lymphocyte by 640 to 2560 µg/ml of Aq- and Me-extract, by 160 to 2560 µg/ml of He-extract compared to the positive (Table 2).

Table 2: In vitro effect of extract of *B. dalzeilii* on proliferation of PHA-activated lymphocytes

Conc. (µg/ml)	Treatments		
	Aqueous Extract	Methanol Extract	Hexane Extract
0	**0,00±0,00**	**0,00±0,00**	**0,00±0,00**
160	**61,11±4,78**	**67,11±4,01**	**150,00±3,07[c]**
320	**72,37±5,70**	**96,93±5,70**	**198,25±1,99[c]**
640	**123,68±12,47[a]**	**146,78±11,05[c]**	**338,01±5,84[c]**
1280	**160,82±6,53[b]**	**208,77±6,35[c]**	**359,50±1,09[c]**
2560	**204,09±27,05[c]**	**108,33±7,01[c]**	**385,96±3,65[c]**
Positive	**47,98±0,44**	**47,98±0,44**	**47,98±0,44**

The data is expressed as Mean ±S.E.M; n=3 wells. [a]P<0.05,[b]P<0.01,[a]P<0.001vspositive.

3.6. Effect of sample extracts on lymphocyte proliferation induced by LPS:

Aq-extract and Me-extract caused profound lymphocyte activation and reached higher proliferation rates than positive or LPS - stimulated cultures ($p<0.05$) in presence of 1280 and 2560 µg/ml of Aq-extract and 2560 µg/ml of He-extract. No statistically significant difference was found between proliferation rates induced by PHA and the ones induced by He-extract (Table 3).

Table 3: In vitro effect of extract of *B. dalzeilii* on proliferation of LPS-activated lymphocytes

Conc. (µg/ml)	Treatments		
	Aqueous Extract	***Methanol Extract***	***Hexane Extract***
0	0,00±0,00	0,00±0,00	0,00±0,00
160	20,62±3,98	12,26±1,14	4,81±1,05
320	44,10±1,01	21,99±4,48	9,05±0,93
640	58,19±4,76	24,40±1,29	11,45±0,30
1280	96,33±2,03^{c}	41,24±4,48	14,89±1,61
2560	110,54±2,59^{c}	84,99±1,46^{c}	25,77±4,10
Positive	45,02±0,99	45,02±0,99	45,02±0,99

The data is expressed as Mean ±S.E.M; n=3 wells$^{a}P<0.05$,$^{b}P<0.01$,$^{a}P<0.001$ vspositive.

3.7. Effect of sample supernatants of stimulated cultures:

The supernatants from culture activated by mature dendritic cells in the presence of the extracts were studied for their mitogenic activity on splenocytes characterizing the presence of IL-2. Proliferation of splenocytes was determined using MTT test. The results demonstrated a significant stimulatory activity of supernatant with Me- and He-extract, while a significant ($p < 0.05$) decreasing in splenocytes proliferation was been observed with Aq-extract (Table 5).

Table 4: Effect of supernatant on splenocytes proliferation characterizing IL-2 production

Conc. (µg/ml)	***Percentage values of splenocytes proliferation in presence of supernatant following the extract concentrations***		
	Aqueous Extract	***Methanol Extract***	***Hexane Extract***
160	-11,88±0,93^{c}	6,87±0,31	7,03±0,15
320	-17,03±1,40^{c}	7,81±0,62	16,41±0,47^{c}
640	-11,72±0,47^{c}	8,13±0,00	26,88±0,00^{c}
1280	-11,25±0,31^{c}	19,53±0,47^{c}	35,00±0,31^{c}
2560	-14,06±3,75^{c}	12,34±1,09^{b}	64,53±0,47^{c}
Positive	7,50±0,01	7,50±0,01	7,50±0,01

The data is expressed as Mean ±S.E.M; n=3 wells. $^{b}P<0.01$, $^{c}P<0.001$ vs positive.

Discussion:

The human immune system evolved to protect the host from potentially pathogenic agents, to eliminate neoplastic cells; and to reject non-self components. It is a complex and highly interactive network of cells and their products which can be modulated by certain agents resulting in immunopotentiation or immunosuppression, resulting in modulation of diseases[16]. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when host defense mechanism has to be activated under the conditions of impaired immune response or when a selective immunosuppression is desired in situations like autoimmune disorders. There are a number of diseases where immunostimulant drugs are required to overcome the immunosuppression induced by drugs or environmental factors and immunosuppressants are required where there is undesired immunopotentiation. There is strong requirement of the drugs which can boost immune system to combat the immunosuppressive consequences caused by stress, chronic diseases like tuberculosis, conditions of impaired immune responsiveness (e.g. AIDS) etc.[17]. In the past a number of drugs with plant or mineral origin have been advocated as means of immunomodulation for various diseased conditions in humans. Most of the plants so far reported with immunostimulatory action have major effect on the non-specific and specfic immunity especially on cells functions. Preparations of plants have the potential to affect almost all aspects of the immune system. The immunomodulatory potential of *B. Dalzeilii* still remains unexplored and this investigation deals with the immunomodulatory screening of different extracts prepared from bark of *B. dalzeilii* plant based on the *in vitro* immunological potential using assays like Nitroblue tetrazolium (NBT reduction), inducible Nitric Oxide Synthase (iNOS), Myeloperoxydase activities and on the modulation of T and B-cell proliferation. Particular, Aq-extract was investigated for its phagocytic activity in vivo. Total 3 extracts were prepared from barks of *B. dalweilii* plant using three different solvent. The sample extracts stimulated macrophage activity (Phagocytosis, NBT, iNOS & Myeloperoxydase) and augmented PHA and LPS induced lymphocyte proliferation.

The process of phagocytosis involves certain body cells, known as phagocytes, which ingest and removes microorganisms, malignant cells, inorganic particles and tissue debris[18]. Phagocytosis and killing of invading microorganisms by macrophages constitute body's primary line of defense. Macrophages are an integral part of the immune system, acting as phagocytic,

microbicidal and tumoricidal effector cells[19]. Through interaction with lymphocytes, macrophages play an important role in the initiation and regulation of immune response. In view of the pivotal role played by the macrophages, Aq-extract of *B. dalzeilii* was evaluated for its in vivo effect on macrophage phagocytic activity as preliminary test. The increase in carbon clearance i.e phagocytic index by Aq-extract reflects the enhancement of phagocytic function of mononuclear macrophage and thus non-specific immunity. This indicates that *B. dalzeilii* could be able to activate macrophages and hence phagocytic assays that could be studied using *in vitro* test.

NBT reduction test is an indirect marker of the oxygen dependent bactericidal activity of the phagocytes and metabolic activity of granulocytes or monocytes[20]. Present results indicate that Me- and He-extracts of *B. dalweilii* plant are capable of stimulating the immune function of macrophages as evidenced by an increase in NBT reduction but out of all samples of Aq-extract. The functional ability of macrophages was evident from increased expression of iNOS that oxidizes L-arginine to citrulline and nitric oxide. The iNOS activity is correlated to bactericidal activity of macrophages and has been documented as a measure of immunomodulatory potential. The sample extracts produced a significant influence on this parameter also with Aq- and Me-extract showing the significant bioactivity.

Myeloperoxidase, a heme protein secreted by neutrophils and macrophages, which uses the oxidizing potential of H_2O_2 to convert chloride ion into hypochlorous acid (HOCl). A potent bactericidal agent, HOCl is a critical component of host defenses against invading bacteria, fungi, and viruses[21]. The increase in the stimulation index of myeloperoxidase by the exposure of *B. dalzeilii* extract indicates enhanced defense capability of these cells to pathogenic organisms. Macrophages incubated with the *B. dalzeilii* extract at different concentrations ranging between 2560 – 160µg/ml, showed a significant activation of macrophages by modulating the activity of various mediators including iNOS, and myeloperoxidase activity. This suggests that *B. dalzeilii* can effectively strengthen innate immunity against foreign particles[22].

Lymphocytes are important cells for the immune system which play an important role in host defense mechanisms for protection from microbial invaders and viral infected cells. When lymphocytes are stimulated with foreign substances, they proliferate and a variety of cytokines are released to induce fundamental defense systems. Different concentration of extracts stimulated profoundly lymphocyte activation by PHA and LPS. The effect of extract was been highly for lymphocytes activated by PHA than with LPS. Proliferation observed with the sample extracts prepared from *B. dalzeilii* plant may be due to an action on T-dependent activation of lymphocyte[23, 24]. The supernatant collected from activated cultures showed a high proliferative activity on splenocytes for Me- and He-extract suggesting the presence of IL-2 [[10]].

Conclusion:

The results obtained suggested that a variation in the bioactivity was observed with respect to the type of solvent extract as well. The Aq-extract, Me-extract and He-extract of the *B. dalzeilii* showed the immunomodulatory potency and was found to be the best immune-potent. However, Aq-extract was found to be without effect on myeloperoxidase and was found to reduce IL-2 production. He-extract was supposed no effect proliferation of lymphocyte B. It is clear from this study that *B. dalzeilii* played an important role in the modulation of the immune response and thus may have applications as an immunomodulatory agent or a drug of choice, effective in treating the diseases where the underlying defect is a T-cell and B-cell deficiency or phagocytic dysfunction. Moreover basic immunomodulatory studies of *B. dalzeilii* on molecular activity will be important.

Acknowledgment:

Authors thank Pr. Zoli, Chief of LASAN, University of Dschang, for providing all the necessary facilities required to carry out this work.

References:

1. Krzystyniak, K., Tryphonas, H., Fournier, M.. *Environmental Health Perspectives*.1995; 103(9): 17-22.
2. Monograph-Momordicacharantia (Bitter melon). Alternative medicine review.2007; 12(4): 360-363.
3. Wagner H. (Ed). Immunomodulatory agents from plants, Basel: Birkhauser, Germany. 1999; 1-35.
4. World Health Organization. Diet, nutrition and the prevention of chronic diseases. Geneva, World Health Organisation. 2003.
5. Ammon H. P. T. Boswellic acid in chronic inflammatory diseases. *Planta Med*. 2006; 72(12):1100-16.
6. Oumar M., Tume C., Monique K. O. and Albert K..In vitro effect of aqueous extract, hexane and m*e*thanol fractions of *boswellia dalzeilii*, hutch (family: *burseraceae*) in immunomodulatory activities of human monocytes / macrophages. *International Journal of Biological & Pharmaceutical Research*.2014; 5(2): 201-209.
7. Boyum, A.. Ficoll Hypaque method for separating mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. Suppl.* 1966; 77.
8. Romani, N., Gruner, S., Brang, D., Kämpgen, E., Lenz, A., Trockenbacker, B., Konwalinka, G., Fritsch, P. O., Steinman, R. M., and Schuler, G. Proliferating dendritic cell progenitors inhuman blood. *J.Exp.Med.* 1994; 180:83-93.
9. Lee S, Park J. Y., Lee W. H., Kim H., Park H. C., Mori K., Suk K.. Lipocalin-2 is an autocrine mediator of reactive astrocytosis. *J Neurosci*.2009; 29:234–249.
10. Kyubum K. and Richard G. Lynch.. A new Non-radioactive Method for IL-2 Bioassay. *Mol. Cells*.1999; 10 (5): 575-578.

11. Archana Ramesh Juvekar, Amolkumar K. H., Sachin Sridhar Sakat, Vivek Avinash Chaughule.. In vitro and in vivo evaluation of immunomodulatory activity of methanol extract of Momordica charantia fruits. *Drug Invention Today*.2009; 1(2): 89-94
12. ManosroiA., Saraphanchotiwitthaya A., Manosroi J. Effects of Pouteria cambodiana extracts on in vitro immunomodulatory activity of mouse immune system; *Fitoterapia*. 2006; 77: 189–193.
13. Waters W.R., Sacco R. E., Fach S. J., Palmer M. V., Olsen S. C.,and Kreeger T. J. Analysis of mitogen-stimulated lymphocyte subset proliferation and nitric oxide production by peripheral blood mononuclear cells of captive elk (*cervuselaphus*).*Journal of Wildlife Diseases*. 2002;38(2): 344–351
14. Choi E. M., Hwang J. K. Effects of methanolic extract and fractions from Litsea cubeb a bark on the production of inflammatory mediators in RAW264.7cells.*Fitoterapia*.2004; 75:141-148.
15. Yan Y., Wanshun L., Baoqin H., Changhong W., Chenwei F., Bing L., Liehuan L. The antioxidative and immunostimulating properties of D-glucosamine; International Immunopharmacology.2007; 7: 29–35.
16. Yamaguchi, H. Immunomodulation by medicinal plant. *AdvExp Med Biol*. 1992; 319: 287-297.
17. Ganju, L., Karan, D., Srivastava, C.K.K., Sawhney, R.C., Selvamurthy, W..*Biomed Pharmacother*.2003; 57:296-300.
18. Salman H, Bergman M, Bessler H, Fenig E, Weiss J, Beilin B, Djaldetti M. Decreased phagocytic capacity of rat peritoneal macrophages following photon abdominal irradiation. *Cancer Letters*. 1999; 147 (1–2):175–179.
19. Son EH, Moon EY, Rhee DK, PyoS..Stimulation of various functions in murine peritoneal macrophages by high mannuronic acid-containing alginate (HMA) exposure in vivo. *International Immunopharmacology*. 2001: 1:147–154.
20. Manosroi A. Saraphanchotiwitthaya A. Manosroi C, J.. Immunomodulatory activities of *Clausena excavate* Burm. f. wood extracts. *Journal of Ethnopharmacology*. 2003; 89: 155–160.
21. Brown KE, Brunt E. M., Heinecke J. W. Immunohistochemical detection of myeloperoxidase and its oxidation products in kupffer cells of human liver. *Am. J. Pathol*. 2001; 159: 2081-2088.
22. Xie OW, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Ding A, Troso T, Nathan C. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science*.1992; 256:225-228.
23. Miller LE. In: Ludke, H.R., Peacock, J.E., Tomar, R.H. (Eds.).Manual of Laboratory Immunology. *Lea and Febiger, London*; 1991; 1–18.
24. Aurason Saraphanchotizitthayan, Kornkanok Ingkanian et Pattana Sripalakit.. Immunomodulating activity of Thai Rejuvenating plants. *Naresuan University journal*. 2007; 15 (3):19-157.