RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.38.47

Immunomodulatory Activity of Ethyl Acetate Extract and Fractions from Leaves of *Crassocephalum bauchiense* (Asteraceae)

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ABSTRACT

Medicinal plants are rich sources of secondary metabolites which are claimed to possess immunomodulatory properties, justifying why the present study was designed to investigate the immunomodulatory potential of *Crassocephalum bauchiense* leaves extracts. Leaves were macerated successively in hexane, ethyl acetate and methanol. The ethyl acetate extract was further fractionated into six fractions (F_1 to F_6) using flash chromatography. The activities of the extract and its fractions were investigated on macrophage NO production model while their proliferative effects were evaluated on macrophages and peripheral blood lymphocytes by the thiazolyl blue (MTT) test. Extract and fractions significantly inhibited NO production by peritoneal macrophages. However, they enhanced macrophage and lymphocyte proliferation *in vitro* at lower concentrations (<64 or <128 μg mL^{-1}). Fraction F_2 significantly increased Delayed-type herpersensitivity reaction (DTH) in mice. This activity was potentiated when the fraction was co-administered with cyclophosphamide. The fraction did not show any activity on antibody production. The total leucocytes count (mainly lymphocytes) was higher in the extract-treated groups when compared to the controls. Fraction F_2 significantly suppressed myelosuppression induced by cyclophosphamide as from 25 mg kg^{-1} of body weight (bw). Oral administration of the fraction caused lethal signs of toxicity from 16 mg kg^{-1} bw. The LD_{50} was estimated at 22 mg kg^{-1} bw. These results indicate that *C. bauchiense* ethyl acetate extract and its fraction F_2 have relatively interesting immunostimulatory effects on lymphocytes. In associated with its kwon antimicrobial properties, one can conclude that this plant species is a candidate for anti-infective phytomedicine preparation.

Key words: Immunomodulatory activity, *Crassocephalum bauchiense,* cell immunity, humoral response, myelosupression

Pharmacologia 4 (1): 38-47, 2013

INTRODUCTION

The immune system dynamism protects living organisms from potentially pathogenic agents including viruses, bacteria, fungi and parasites. It eliminates neoplastic cells and rejects non-self-components (Juvekar *et al*., 2009). Weakness of the immune system increases susceptibility to infections. Many herbal products have been evaluated for their capacity to modulate the immune system (Biella *et al*., 2008; Sharififar *et al*., 2009). They act by stimulating both specific and non-specific immunity (Mediratta *et al*., 2002). These actions may concern both humoral and cell mediated immunity or only one of these mechanisms (Bafna and Mishra, 2004). The high incidence of multidrug-resistant microorganisms and the slow rate of new antibiotic discovery suggest that alternative strategies be found to enhance the existing antibiotics. *Crassocephalum bauchiense* (Asteraseae) is used in folk medicine in the West Region of Cameroon to treat gastrointestinal infections. Earlier studies on this plant reported *in vitro* and *in vivo* antibacterial activity (Mouokeu *et al*., 2011). A dual antimicrobial activity and stimulatory action of the immune system may be useful to target resistant bacteria where conventional antibiotics are failing. The present study was thus designed to investigate the immunomodulatory potential of ethyl acetate extract and fractions from *Crassocephalum bauchiense* leaves.

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MATERIALS AND METHODS

Plant material: *C. bauchiense* leaves were collected in Dschang, West Region of Cameroon. Botanical identification was done at the National Herbarium in Yaoundé (Cameroon) by referring to the voucher specimen number 7954/SRF/Cam.

Preparation of the plant extract: *C. bauchiense* leaves were dried at room temperature (20±2°C) and ground into powder. The dried powdered leaves were macerated in ethyl acetate at room temperature for 48 h. The extract obtained was dried at 40°C using a vacuum evaporator under reduce pressure. Fifty grams of this extract was submitted to flash chromatography using silica gel (Merck, 70-230 mesh, Sigma Aldrich, Germany) as stationary phase. The elution was done using increasing solvent polarity made of hexane, ethyl acetate and methanol mixtures. Fractions of 500 mL each were collected and evaporated under vacuum, then combined on the basis of their thin layer chromatography profiles into six fractions denoted F_1 to F_6. Phytochemical analysis of the extract and fractions was performed following classical methods described by Harbone (1973).

Assessment of the *in vitro* immunomodulatory activity

Preparation of macrophage suspension: Peritoneal macrophages were obtained from rats prior injected with 2 mL of New Born Calf Serum (NBCS), intraperitoneally, two days before. Peritoneal exudates were collected in RPMI-1640 culture medium supplemented with 10% NBCS, 2 mM L-glutamine, 100 U mL^{-1} penicillin and 100 g mL^{-} gentamycin (RPMI 10%) were centrifuged at 1000 rpm and 25°C for 20 min. The erythrocytes were destroyed using 10 mM EDTA at 4°C for 30 min (hypotonic lyses). The mixture obtained was centrifuged at 1800 rpm for 15 min. All pellets were washed twice with RPMI 10%. The cell concentration was determined using Malassez chamber and cell viability was assayed by trypan-bleu exclusion dye technique. Macrophages were then adjusted to 5×10^6 cells mL^{-1} (Chaves *et al.*, 2007).

Preparation of lymphocyte suspension: Peripheral blood lymphocytes were isolated from blood of adult rats by Ficoll Hypaque Density Centrifugation. Lymphocytes were separated at interface and washed thoroughly with phosphate buffer solution, viability was checked by Trypan dye exclusion test using Malassez chamber and the cell suspension was adjusted to 5×10^6 cells mL^{-1} in RPMI 10% (Chaves *et al.*, 2007).

Lymphocyte and macrophage cultures: One hundred microliters of RPMI 10% were dispensed into each well of 96 microtitre plates. The stock solution of *C. bauchiense* extract or fractions was dissolved in 5% tween 80. A serial two-fold dilution of the extract was performed to obtain a final concentration range of 256 to 8 µg mL^{-1}. One hundred microliters of the cell suspension were introduced into each well to yield a final volume of 200 μL $well^{-1}$. Phytohemaglutinin A (64 μg mL^{-1}) was used as positive control while 5% tween 80 was used as negative control. Plates were incubated at 37°C, 5% CO_2, 80% humidity on coleparmer CO_2 incubator for 24 h (macrophages) and 72 h (lymphocytes) (Chaves *et al.*, 2007).

Determination of nitric oxide production: The production of nitric oxide (NO) was estimated from the accumulation of nitrite (NO_2^-) in supernatant drawn from macrophage culture using Griess reagent (Reis *et al.*, 2001; Chi *et al.*, 2003). Briefly, equal volumes of samples and Griess reagent (1% sulphanilamide and 0.1% naphthylamine in 2.5% phosphoric acid) were mixed and incubated at room temperature for 10 min. The absorbance values were measured at 570 nm using a microplate reader. Results fraction were expressed as percentage inhibition of NO production due to treatment with extract or fractions were converted to IC_{50} that represents the concentration (μg mL^{-1}) of the extract or fraction responsible for the inhibition of 50% of NO production.

Effect of *C. bauchiense* ethyl acetate extract and its fractions on macrophage and lymphocyte proliferations: Upon incubation, 25 μL of 3-[4,5-dimethylthiazol-2-yl] 2,5 diphenyltetrazolium bromide (MTT) (Sigma Aldrich, Germany) were added to each well followed by four additional hours of incubation at 37°C. The reaction was stopped by the addition of 50 µL of 20% SDS in dimethylformamide pH 4.7. Absorbance was recorded at 550 nm (Chaves *et al.*, 2007). Percentage of proliferation obtained with medium containing 5% tween 80 was established as 100% and used as reference. Percentage of proliferation obtained with extract was calculated by dividing the absorbance of the experimental well by the absorbance of the control (5% tween 80, Sigma Aldrich, Germany) and multiplying by 100. When the percentage obtained was significantly higher than 100%, the substance was considered stimulatory. Likewise, when it was significantly lower than 100%, the substances were considered inhibitory of cell proliferation (Chaves *et al.*, 2007).

***In vivo* immunomodulatory activity of fraction F_2**

Animals: *Swiss* albinos female mice (18-25 g) were bred and maintained under standard laboratory conditions (25±2°C), 12:12 h (photoperiod). They were fed with a standard diet. Food and water were given *ad libitum* to all animals used for the experiments. The study was conducted according to the ethical guidelines of Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

Delay type hypersensitivity (DTH) reaction: Mice were divided into four groups of ten each. The control

group received vehicle (5% tween 80), while mice in the treatment groups were administered with the fraction F_2 (25, 50 and 200 mg kg^{-1} bw.) daily for 14 days. On the 7th day, animals were immunized subcutaneously with 0.1 mL of bovine serum albumin (BSA, 1 mg mL^{-1}) prepared with Freud adjuvant in the plantar region of right hind foot paw and challenged on day 14th by subcutaneous injection of the same amount of antigen and PBS in left hind paw in same volume. Before immunization, five animals in each group were immunosuppressed by intraperitoneal injection of 30 mg kg^{-1} cyclophosphamide for three consecutive days. Food pad reaction was assessed on the 8th and 15th day, in terms of increase in the thickness of foot pad as a result of hypersensitivity reaction due to oedema, the thickness of the right hind foot pad was measured using vernier calliper. The foot pad reaction was expressed as the difference in the thickness (in mm) between the right foot pad injected with BSA and the left foot pad injected with PBS (Nworu *et al.*, 2007).

Assessment of antibody production: Forty mice were divided into three test groups and one control group. They were immunized by intramuscular injection (i.m.) of 0.1 mL of 1 mg mL^{-1} BSA on day 0 and challenged by similar injection of the same amount on day 7. Primary antibody titre was determined on day 7 (before the challenge) by the agglutination technique (Kanjwani *et al.*, 2008). Fraction F_2 (25, 50 and 200 mg kg^{-1}) was administered 7 days prior to immunization and continued daily for 7 days after the challenge; tween 80 (5%) was used as control. Seven days after immunization, blood samples were obtained by cardiac puncture into test tubes and allowed to clot. For each sample, 50 μL serum was obtained after centrifugation and serially diluted two-fold in 96 well microtitre plates using Phosphate Buffer Saline (PBS) as control. The diluted sera were challenged with 50 μL of 0.5 mg mL^{-1} BSA and then incubated at 37°C for 1 h. The highest dilution giving rise to visible agglutination was taken as antibody titre. Antibody titre was expressed in graded manner, the minimum dilution (1/2) being ranked as 1.

Leucocyte mobilization and cyclophosphamide induced myelosuppression: Cyclophosphamide induced myelosuppression was studied according to the method described by Manjrekar *et al.* (2000) with slide modifications. Mice were divided into four groups consisting of ten animals each. Group I received 5% tween 80 (p.o) and served as control. Mice in treatment groups (II, III and IV) were given the fraction F_2 (25, 50 and 200 mg kg^{-1} bw) daily for 16 days. On days 17, 18 and 19, five animals of each group were immunosuppressed with cyclophosphamide (30 mg kg^{-1}, ip) one hour after administration of the extract. On day 20 blood samples of the animals were collected separately and blood cells were analyzed. Immediately after the blood collection, the liver, spleen and kidneys were carefully dissected out, blotted, observed macroscopically and weighed immediately using a Sartorius electronic balance. The relative organ weight (Row) of each animal was then calculated as followed.

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on day of sacrifice (g)}} \times 100$$

Acute toxicity study: Eighty (80) male and female *Swiss* albino mice, weighing 20-25 g, of 8 to 10 weeks old were used for the acute toxicity study. They were randomly distributed into one control group and seven treated groups, containing ten animals per group (5 females and 5 males) and were provided with standard animal food and water *ad libitum*. The mice were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After allowing the animals to fast over-night, the control group received 0.1 mL of 5% Tween 80 solution (oral gavage). Each treated group received one of fraction F4 doses as follows: 12, 14, 16, 18, 20, 22 and 24 g kg^{-1} bw. The animals were observed continuously for the first 4 h, then hourly for the next 24 h, then after 6 hours for 48 h and finally daily for 14 days after administering the extract to observe any changes in general behavior or other physiological activities. During this period food intake and weight gain were reported (Burger *et al.*, 2005).

Statistical analysis: The experimental results were expressed as the Mean±Standard Deviation (SD). Group comparisons were performed using One Way ANOVA followed by Waller-Duncan Post Hoc test. A p value of 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening: The results of the phytochemical screen reported in Table 1 indicated the presence of phenols, alkaloids, flavonoids, tannins, triterpenes and sterols in the extract. These chemical groups of compounds were relatively present in the fractions.

In vitro immunomodulatory activity

Effect of *C. bauchiense* extract and its fractions on nitric oxide production: *C. bauchiense* ethyl acetate extract and fractions showed significant inhibitory activities on NO production by peritoneal macrophages (Table 2). Fractions F_2, F_3 and F_4 revealed greater activities compared to the extract while fractions F_5 and F_6 were found to be inactive.

Table 1: Phytochemical screening of the *C. bauchiense* ethyl acetate extract and fractions

Classes of compounds	Extract	Fraction F_2	Fraction F_3	Fraction F_4	Fraction F_5	Fraction F_6
Phenols	+	+	+	-	-	+
Alcaloïds	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
flavonoïds	+	-	+	-	-	-
Tanins	+	+	+	-	-	+
Triterpenes	+	+	-	-	-	-
Sterols	+	+	+	+	+	-

+: Present, -: Absent

Table 2: Inhibitory concentrations 50 (IC_{50}) of ethyl acetate extract and fractions from *C. bauchiense* on nitric oxide production by macrophages

Samples	IC_{50} ($\mu g\ mL^{-1}$)
Crude extract	90
Fraction F_2	<8
Fraction F_3	<8
Fraction F_4	6.65
Fraction F_5	398.08
Fraction F_6	310.75

Table 3: Effect of the fraction F_2 of *C. bauchiense* extract on antibodies response in mice

	Antibodies response (Antibodies titre ±SD)	
Doses (mg kg^{-1})	Primary response	Secondary response
0	31,00±0,00^a	36,00±0,00^a
25	31,33±0,33^a	36,50±0,50^a
50	31,00±0,00^a	36,33±0,33^a
200	31,50±0,50^a	36,25±0,22^a

Values are expressed as Mean±SD, n = 5; In the same column, values bearing same superscript letters are significantly equal according to Waller-Dunkan test (at $p < 0.05$)

Effect of extract and fractions on macrophage and lymphocyte proliferations: *C. bauchiense* ethyl acetate extract and its fractions revealed stimulatory activities on both macrophage and lymphocyte proliferations that varied with the tested concentrations (Fig. 1, 2). These activities were greater than that of phytohemaglutinin A used as positive control (Fig. 1, 2). Except for the fraction F_2, all the tested concentrations revealed stimulatory activities, the highest proliferative activities with the extract and other fractions were achieved at lower concentrations.

***In vivo* immunomodulatory activity of fraction F_2**

DTH response in BSA immunized mice: Fraction F_2 significantly ($p<0.05$) potentiated DTH response in terms of increase in the mean difference of paw thickness when compared with control group in either the primary or the secondary challenge (Fig. 3). In groups of mice treated with cyclophosphamide i.e. immunosuppressed groups, this fraction was found to be more active.

Detection of serum antibody response to bovine serum albumin (BSA): All the tested doses of fraction F_2 showed neither activity on antibody production nor the primary and/or secondary antibody titre (Table 3).

Leucocyte mobilization and cyclophosphamide induced myelosuppression: Fraction F_2 showed no significant effect on total red blood cells (RBC_S) count, but a significant increase in hematocrit was observed at all the doses compared to control group (Table 4). Moreover, significant dose-dependent increases of total white blood cells (WBC_S) and lymphocyte counts were observed. Independently of dose, there were also significant increases of RBCs, WBCs, lymphocytes, macrophages and neutrophiles when the fraction was administered in the presence of cyclophosphamide, an immunosuppressive agent.

Relative organ weight: In the presence of immunosuppressive effect of cyclophosphamide, the fraction F_2 significantly increased the relative organ weight of spleen and liver at doses of 25 and 50 mg kg^{-1} bw (Table 5). However, no significant effect on liver, spleen and kidneys was observed when this fraction was administered in the absence of cyclophosphamide.

Acute toxicity: From 16 g kg^{-1} bw, animals treated orally with the fraction F_2 exhibited toxicological signs such as depression in aggressiveness, sensitivity to external stimulus and in locomotion as compared to control. Food intake and weight gain was not affected during the experiment time. The oral LD_{50} of fraction F_2 was found to be 22 g kg^{-1} bw.

Substances that are able to activate an impaired host immune system can be helpful to the conventional (Wagner, 1984) and this may explain the growing interest to phytomedicine with immunomodulatory properties (Tiwari *et al.*, 2004). The main objective of this study was to investigate the immunomodulatory effects ethyl acetate extract and fractions from *Crassocephalum bauchiense* leaves.

In vitro immunomodulatory assay showed that the ethyl acetate extract of *C. bauchiense* leaves and its fractions stimulate lymphocyte and macrophage proliferation *in vitro*. Thus, the ethyl acetate extract of *C. bauchiense* may contain costimulatory molecules which act as direct mitogen on lymphocytes and macrophages (Redondo, 2000). These results were obtained without any lectin in the culture medium, indicating that the extract may contain lectins (Chaves *et al.*, 2007). The proliferative

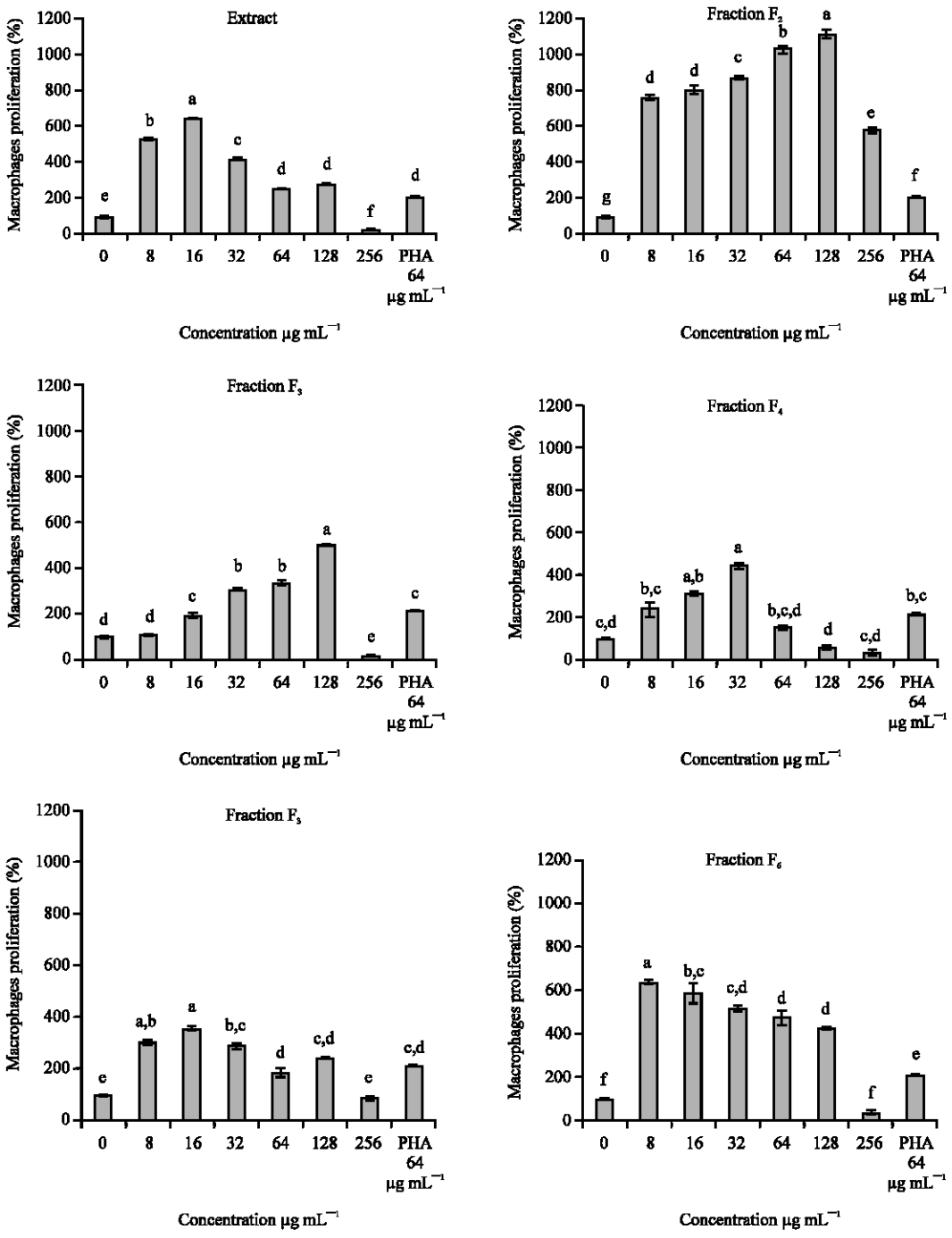

Fig. 1: Effect of *C. bauchiense* ethyl acetate extract and its fractions on macrophages proliferation. Values of extract/fraction concentrations bearing different superscript letters are significantly different according to Waller-Dunkan test (at $p<0.05$); PHA: phytohemaglutinin A

activity of the extract and its fractions was found to decrease as the concentration increased. This suggests that the cytotoxic activity could be expected at higher concentrations. However fraction F_2 did not express any cytotoxicity effect showing that best activity could be expected at high concentrations.

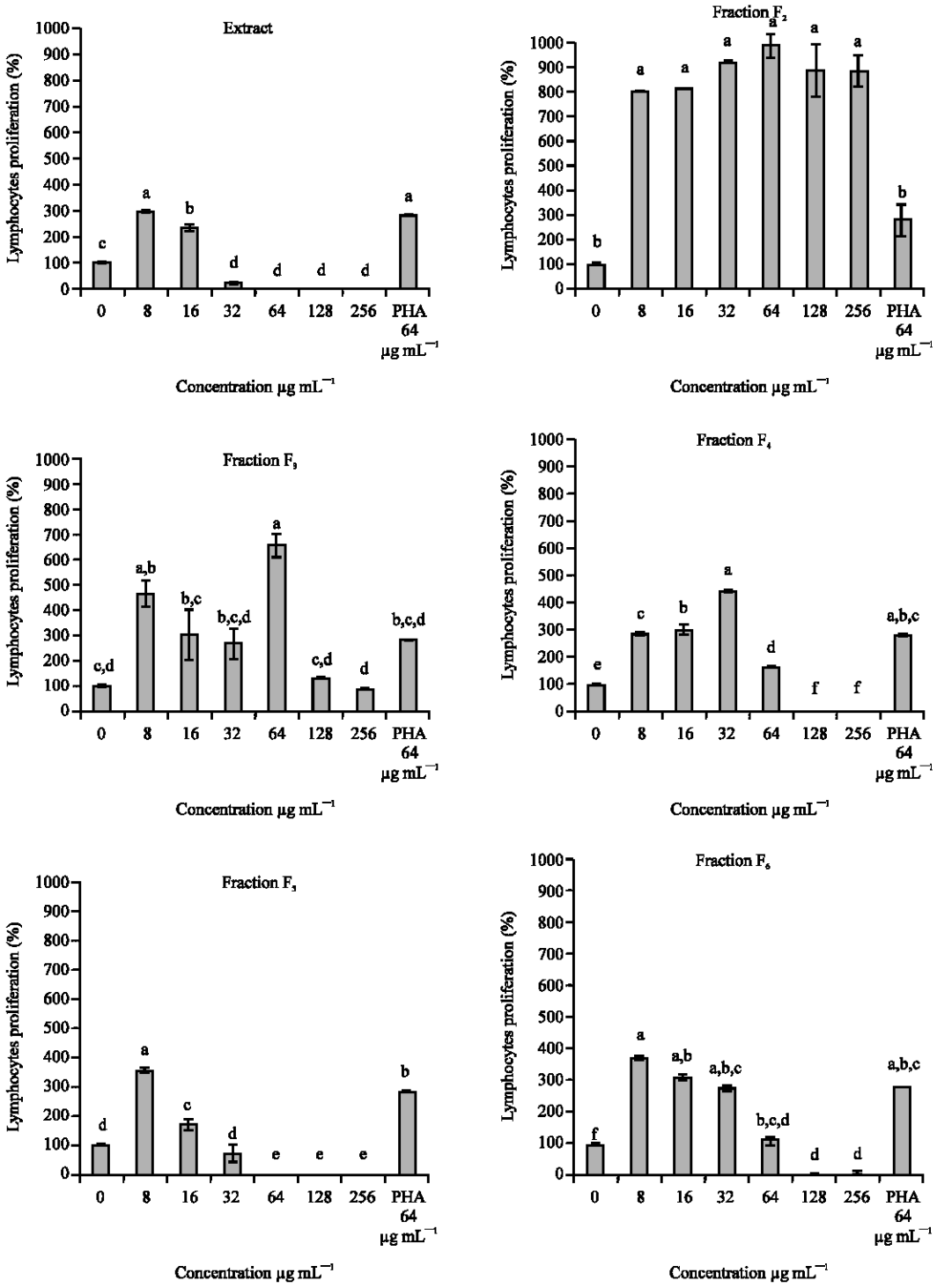

Fig. 2: Effect of *C. bauchiense ethyl acetate* extract and its Fractions on lymphocytes proliferation. Values of extract/fraction concentrations bearing different superscript letters are significantly different according to Waller Dunkan test (at $p<0.05$); PHA: phytohemaglutinine A

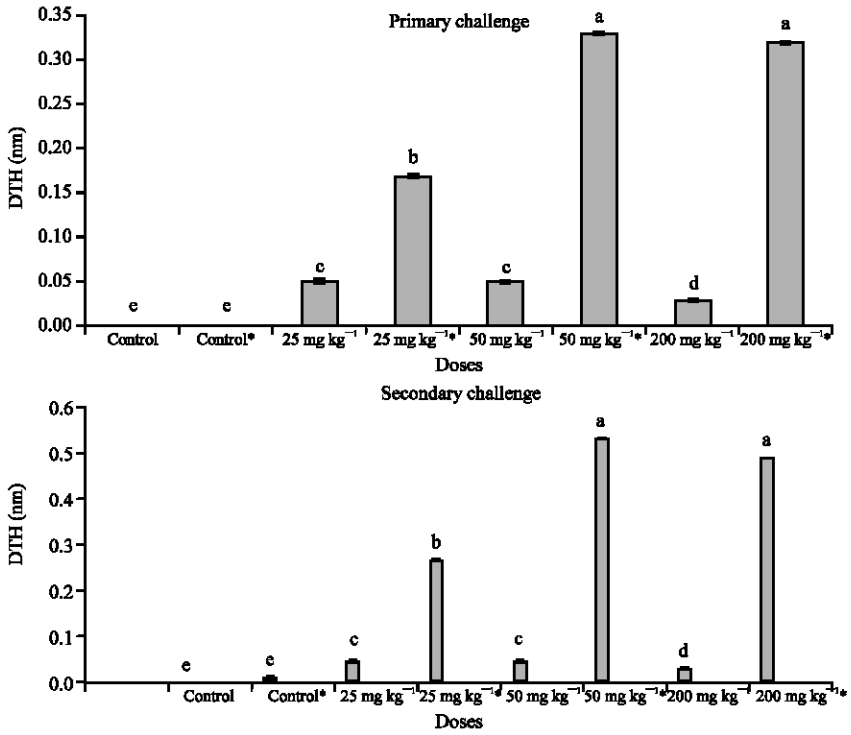

Fig. 3: Effect of fraction F_2 of *C. bauchiense* extract on delayed type hypersensitivity reaction in mice, DTH: Delayed-type herpersensitivity, Values are expressed as Mean±SD, n = 5; * = Cyclophosphamide treated groups (positif control); values of fraction concentrations bearing different superscript letters are significantly different according to Waller-Dunkan test (at $p<0.05$)

Table 4: Effect of fraction F_2 of *C. bauchiense* extract on leucocytes mobilization and cyclophosphamide induced myelosuppression

Doses (mg kg^{-1})	RBC ($\times10^3$/mm^3)	WBC (mm^3)	Lymphocytes (%)	Macrophages (%)	Neutrophiles (%)	Basophiles (%)	Eosinophiles (%)	Hematocrit (%)
0	4000±144.00^a	169±0.00^c	46.33±4.6^c	5.67±3.20^a	45.67±3.51^a	1.33±0.57^a	1.00±0.00^a	29.00±5.35^b
25	4045±77.00^a	198±10.00^b	54.75±3.77^b	7.25±1.50^a	39.00±6.63^a	1.25±0.04^a	0.75±0.01^a	34.75±8.18^a
50	4205±28.00^a	202±9.00^a	57.00±2.45^a	6.25±2.63^a	45.00±4.55^a	0.75±0.05^a	1.00±0.00^a	38.00±2.16^a
200	4121±61.00^a	201±11.00^a	58.50±2.08^a	6.50±1.91^a	43.75±3.86^a	0.75±0.00^a	0.80±0.00^a	38.75±7.13^a
0*	3550±25.00^b	127±20.00^d	37.00±0.00^c	4.00±0.00^b	26.00±0.00^b	1.00±0.00^a	1.00±0.00^a	15.08±1.91^b
25*	4250±48.00^a	199±15.00^b	54.50±4.18^a	6.50±2.08^a	37.75±5.05^a	1.00±0.00^a	0.75±0.01^a	36.25±3.92^a
50*	4053±53.00^a	205±12.00^a	48.33±1.15^b	6.33±1.52^a	44.33±1.52^a	0.73±0.00^a	0.80±0.00^a	31.67±2.00^a
200*	4063±28.00^a	200±10.00^a	52.00±2.45^{a,b}	6.00±2.14^a	41.50±1.00^a	1.00±0.00^a	1.00±0.00^a	34.08±4.93^a

Values are expressed as Mean±SD, n = 5, WBC= White Blood Cells, RBC= Red Blood Cells, * = Cyclophosphamide treated groups, For the same group, values bearing different superscript letters are significantly different according to Waller-Dunkan test (at $p<0.05$)

Table 5: Effect of fraction F_2 from *C. bauchiense* ethyl acetate extract on relative organ weight

Doses (mg kg^{-1} bw)	Liver	Spleen	Kidneys
0	8.31±0.40^{a,b}	0.80±0.20^{bc}	2.11±0.10^a
0*	6.24±0.30^c	0.52±0.00^d	2.00±0.10^a
25	8.52±1.42^{a,b}	0.91±0.22^{a,b}	2.10±0.23^a
25*	8.90±0.52^{a,b}	0.80±0.10^{bc}	2.02±0.00^a
50	8.000±0.63^{a,b}	0.72±0.11^{bc}	2.12±0.20^a
50*	9.23±0.52^a	1.34±0.00^a	2.01±0.30^a
200	8.30±0.50^{a,b}	0.90±0.12^{a,b}	2.13±0.20^a
200*	7.60±0.40^c	0.75±0.21^c	2.00±0.20^a

* = Cyclophosphamide treated groups, Values are expressed as Mean±SD, n = 5; In the same column, values bearing different superscript letters are significantly different according to Waller-Dunkan test (at $p< 0.05$)

This result is relevant since lymphocytes participate principally in innate (monocytes and NK cells) and acquired (T and B cells) immune defenses. From previous studies, natural herbal products activity against pathogens and tumors are directly correlated to their ability to stimulate lymphocyte proliferation (Chaves *et al*., 2007). Also, activation of macrophages is followed by an increase in their phagocytic activity and the release of various molecules such as cytokines and others reactive intermediates that permit non-specific immune responses (Lowenstein and Snyder, 1992). In this study, the inhibition of the NO production is

important in three aspects. First, massive amounts of NO generated by activated macrophages may be responsible not only for immunological defense against tumor cells and pathogens, but also may cause injury in cells, damaging several tissues. In addition, the excess of NO is implicated in sepsis and endotoxic shock during an inflammatory process (Chi *et al.*, 2003). Secondly, if there is a simultaneous production of superoxide and NO, the speed of peroxynitrite formation by macrophages is rapidly increase (Rimbach *et al.*, 2000). Peroxynitrite can provoke protein nitration, DNA-strand breakage and guanine nitration, which may cause mutagenesis. NO causes diverse chemical reactions in biological systems, including lipid peroxidation and promotes oxidative damage to macromolecules and tissues. In addition, it exhibits a direct cytotoxic action in cells in culture (Velazquez *et al.*, 2003). To prevent cell damages by NO production, natural product from medicinal and dietary plants are good candidates. Thirdly, as reported elsewhere, free radical and NO or their derivatives are involved in carcinogenesis (Maeda and Akaike, 1998). The events involving chronic inflammation may trigger carcinogenesis. Thus, an intervention against NO overproduction in chronic inflammation could avoid the development of tumors (Maeda and Akaike, 1998). Nitric oxide is an inflammatory mediator produced in a variety of tissues by nitric oxide synthase (NOS) during a catalytic transformation of L-arginine to citruline (Reis *et al.*, 2001). Taken together, these data suggest that *C. bauchiense* extract could be an anti-inflammatory agent. Further studies to determine the anti-inflammatory activity of this plant, are necessary to confirm or infirm this hypothesis.

Results of the *in vivo* investigation showed no effect of the fraction F_2 on antibody titre in response to BSA, but it acts by increasing DTH response reflecting an overall elevation of cellular immune response. DTH is antigen specific response and causes erythema and induction at the site of antigen infection in immunized animals. It is characterized by an influx of immune cells at the site of infections and an apparent induction within 24 to 72 h. T-cells are required to initiate the reaction (Poulter *et al.*, 1982). Significant increase in DTH response indicates that the extract has stimulatory activity on T-lymphocytes especially those having CD4 or CD8 receptors and therefore the fraction F_2 of *C. bauchiense* extract acts on cell mediated immunity. No increase in antibody titre indicates that the fraction does not affect the activity of B-lymphocytes. Similar results were obtained with the effect of the fraction on leucocyte mobilization and cyclophosphamide induced myelosuppression. Indeed, the administration of *C. bauchiense* extract either in the presence or absence of cyclophosphamide significantly ameliorated the total WBC_S count. This indicates a possible action of the extract to enhance immune cells components and also the restoration of the myelosuppressive effects induced by cyclophosphamide. Similar results were reported by Shah *et al.* (2008).

Lymphocytes T and their products such as lymphokines are responsible for Cell-mediated immunity. This type of immunity is important in anti-infective defense of the organism, tumour immunity and delayed-type hypersensitivity reactions (Miller and Peacock, 1991). This immunostimulatory activity constitutes an alternative system which appears to strengthen body's defense mechanism first which in turn will tackle the diseases. The cells proliferative activity both *in vitro* and *in vivo* could be attributed to the presence of phenols, flavonoids, saponins and alkaloids (Lee *et al.*, 2005; Dashputre and Naikwade, 2010).

Administration of fraction F_2 in the presence of cyclophosphamide increased spleen and liver weights in mice at the evaluated doses. It has been postulated that liver enlargement can be associated to physiological adaptation to stress, metabolic abnormalities, toxic effects, inflammatory processes or proliferative diseases (Williams and Iatropoulos, 2002). Thus, the observed increase of the spleen and liver could be related to the proliferative activity.

In acute toxicity study fractions F_2 exhibited toxicological signs in mice such as depression in aggressiveness, sensitivity to external stimulus and in locomotion. These observations suggest that this fraction may have a depressant or sedative effect on the central nervous system (Tamokou *et al.*, 2011). The LD_{50} was found to be 22 g kg^{-1}. According to Hodge and Sterner (1943), substances with LD_{50} value of 15000 mg kg^{-1} bw/oral route are regarded as relatively harmless. The high LD_{50} obtained is an indication that the extract could be administered with a high degree of safety.

CONCLUSION

The results of this study revealed that *C. bauchiense* ethyl acetate extract and fractions has immunomodulatory effect on cell mediated components of the immune system. These results, associated to the antimicrobial properties previously demonstrated may explain the use of this plant species for the treatment of gastrointestinal infections.

ACKNOWLEDGMENTS

Authors acknowledge the financial support of Aires Sud (Appuis Intégrés pour le Renforcement des Equipes Scientifiques du Sud), *Ministère Français des Affaires Etrangères et Européennes*, France, under the grant No 7082 and the National Herbarium of Yaoundé, Cameroon for plant identification.

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