Research Article

Effects on the Phagocytosis Modulation of Roots Extract and Flavonoids from *Allexis batangae* (Violaceae)

Nganso Ditchou Yves Oscar[1,*], Nkwengoua Tchouboun Zondegoumba Ernestine[2], Ndogo Eteme Olivier[2], Soh Desire[3], Nguefeu Nkenfou Celine[4,5], Nyasse Barthelemy[2]

[1]Department of Chemistry, Faculty of Science, University of Maroua, P.O Box 814, Maroua, Cameroon.
[2]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O Box 812, Yaounde, Cameroon.
[3]Department of Chemistry, Higher Teachers Training College, University of Bamenda, P.O Box 39, Bambili, Bamenda, Cameroon.
[4]Department of Biological Science, Higher Training Teaching College of Yaounde I, Molecular Biology Center of Yaounde, P.O Box 812, Yaounde, Cameroon.
[5]Systems Biology Laboratory, "Chantal Biya" International Reference Centre for Research on HIV/AIDS Prevention and Management (CBIRC), Yaounde, CBIRC. PO Box 3077 Yaounde, Cameroon.

***Corresponding author's E-mail:** nganso_yves@yahoo.fr

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ABSTRACT

In this work, we studied the immunomodulation activity of *Allexis batangae* infused roots extract (AIRE) and compounds from this extract, with respect to phagocytosis. AIRE was obtained by infusion of dry bark powder in water and the compounds were isolated after fractionation of AIRE in solvent systems followed by column chromatography. Four flavonoids were obtained from this plant, 4,4''-dimethoxylophirone A (1), 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one (2), jaceosidine or 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (3) and corniculatusine or 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-methoxy-4H-1-Benzopyran-4-one (4). The structure elucidation was based on analysis of spectroscopic data including 1D- and 2D-NMR. The capacity of GLIBE and these flavonoids to modulate phagocytic activity of polymorphonuclear (PMNs) cells was evaluated *in vitro* against *Candida albicans*. The stimulation percentage of PMNs ranged between 4.25 and 42.55 % with AIRE, 14.89, 46.80, 4.25 and 59.57 %. The results showed that AIRE liberates the glutathione particles from animal's liver and enhance the phagocytic activity by stimulating the reticuloendothelial system. The phagocytic index obtained ranged between 0.020±0.004 and 0.074±0.006. These results valorize *Allexis batangae* extracts like possibilities of drugs against immune system dysfunction.

Keywords: *Allexis batangae*; bioactive compounds; immunomodulation; phagocytic activity.

INTRODUCTION

The body has immune agents to defend against invading pathogens and to eliminate diseases[1]. Susceptibility to microbial, allergic and other disorders is higher in the presence of a compromised immune system that results in a state of immunodeficiency. Under such conditions, the ability of the immune system to defend the body against infectious diseases is compromised or completely absent[2]. Immunomodulatory therapy is recognized as an alternative to conventional chemotherapy for various diseases, involving an altered immune response of the host[3-5]. The concept of immunomodulation involves nonspecific activation of the function and efficacy of macrophages, granulocytes, complement natural killer cells and lymphocytes as well as the production of different effect or molecules generated by activated cells. These nonspecific effects are expected to confer protection against various pathogens, including bacteria, viruses, fungi, etc., and provide an alternative to conventional chemotherapy[1,6]. Immunostimulators are known to support T-cell functions, activate macrophages, granulocytes, complement cells and natural killer cells, and affect the production of different effector molecules generated by activated cells [7].

Several currently available immunomodulators, including levamisole, glucans, telons, L-fucose, as well as the bacterium *Cornybacterium parvum*, have one or more adverse effects such as fever, neutropenia, leukopenia and sometimes allergic reactions [8]. As a result, screening for new immunomodulators is urgently needed [9]. The search for more effective and safer immunomodulatory agents is becoming a major area of concern around the world [10]. Agents that regulate host defense mechanisms in the presence of altered or exaggerated immune reactivity may provide supportive treatment to conventional chemotherapy [11,12].

Previous studies have shown that some medicinal plants have immunomodulatory activities [13-15]. Several plants used in traditional medicine can modulate the body's immune system [16]. Herbal preparations originally used in traditional medicine systems, are currently being studied and tested in various pathophysiological states [17]. They are an important source of substances that constitute non-specific immunomodulation essentially of granulocytes, macrophages, natural. Killer and complementary

functions[18]. They are traditionally used to treat immune disorders, such as inflammatory and autoimmune diseases. *Allexis batangae* is used in Cameroonian pharmacopoeia to treat many diseases. A stem bark decoction of this plant is traditionally used to treat stomach upset, gastrointestinal infections, malaria, skin diseases, gynecological disorders and pregnancy problems. The leaves are used for the treatment of swelling, edema of venomous bites, gout, febrifuges. It is also used as a genital stimulant, laxative and for the treatment of venereal diseases. Leaf sap is used for the treatment of eyesight, as an analgesic and to treat heart disease [19]. To the best of our knowledge, no scientific study concerning the immunomodulator effects of *Allexis batangae* have been reported in literature. This study was undertaken to evaluate the capacity of root extracts and flavonoids from this plant to modulate the phagocytic activity.

MATERIALS AND METHODS

General experimental technique

After drying, the roots of *Allexis batangae* were crushed using a crushing machine. The maceration of the powder in CH_2Cl_2-CH_3OH (1:1) was done in a tightly sealed 25 L can.

A MARQLUTAN GM-300P electronic scale weighed the raw extract and the different masses of the fractions.

Flash chromatography was performed using a VELP Scientifica vacuum cleaner, a Buchner and a vacuum flask;

A Büchi brand Heidolph WB 200 rotary evaporator was used to separate the extract from the solvent;

Column chromatographies were carried out in a column 3 cm in diameter and 60 cm long and a small column 2 cm in diameter and 50 cm in length.

1D (1H) and 2D (HSQC, HMBC,) NMR spectra were obtained from a 600 MHz spectroscopic apparatus. Column chromatography was performed on silica gel 60 [(0.2-0.5 mm) and (0.2-0.063 mm)] mesh (Sigma-Aldrich, Germany). Pre-coated silica gel 60 F254 thin layer chromatography (TLC) plates (Merck, Germany) were used for monitoring fractions and spots were detected with UV light (254 and 365 nm) and then sprayed with 30 % sulphuric acid (H_2SO_4) followed by heating to 110 °C.

Plant collection and identification

Allexis batangae was harvested on 7th June 2014 at Bidou II, 20 km from the town of Kribi (South Cameroon) under the leadership of Mr. NANA (Botanist). The identification was carried out at the National Herbarium of Cameroon by Mr. NANA in comparison with specimen number 31839/HNC. Identified in Gabon and Southern Cameroon (Kribi) in the Kienke forest, it is a small Shrub up to 6 m tall, with a pale brown smooth stem and small leaves. The flowering is done on the stem. It has pedicel 15 mm long[19].

Extraction and isolation

Dried and powdered root of *Allexis batangae* (1 kg) was extracted with CH_3OH (3 L) at room temperature and evaporated under vacuum to yield a crude extract. 100 g of this extract was dissolved in CH_3OH-H_2O (8:2) and partitioned with *n*-hexane (3×150 mL) and ethyl acetate (3×200 mL). The ethyl acetate portion (35 g) was subjected to column chromatography over silica gel eluting with gradients of CH_2Cl_2/CH_3OH to produce 79 fractions of 250 mL each. These fractions were combined based on their TLC profiles into 3 major fractions: A (5.2 g, 1–40); B (3.6g, 41–55); C (3g, 56–79). Fractions A (CH_2Cl_2/CH_3OH 50:1); B (CH_2Cl_2/CH_3OH 40:1); C (CH_2Cl_2/CH_3OH 30:1).

Phytochemical screening

Qualitative phytochemical tests were done on the crude extract according the standard procedures described in literature by Sofowora *et al*. [20] and Harbone *et al*. [21].

Phagocytic activity

Ethical Approval

The Institutional Animal Ethics Committee (878/ac/05/ CPCSEA/008/201) of the Raghavendra Institute of Pharmaceutical Research Education and Research, Anantapur, Andhra Pradesh, India, approved the experimental protocol for the development of animal. The study protocols were approved by the Research Ethics Board of the Center for Management of Research and Development, University of Port Harcourt, with ref. No: UPH/CEREMAD/REC/04. The rats in the study were handled humanely in accordance with the ethical and regulatory rules governing the use of research animals, approved by the University.

In vitro phagocytic activity of polymorphonuclear (PMNs) cells

The effect of *Allexis batangae* extracts on the immunomodulation of human polymorphonuclear cells was carried out according to the method described by Ponkshe *et al.* [22]. From a 24-hour *Candida albicans* culture on a Sabouraud Dextrose agar plate, the inoculum was prepared by suspending a pure yeast colony in 1 mL of 1:4 human serum in NaCl saline at 0.9 %. The concentration of the cell suspension was adjusted to $1x10^8$ by counting under the microscope. Human blood (0.2 mL) was obtained by a finger prick method on a sterile glass slide. Slides in triplicate were incubated at 35 °C for 25 minutes to allow coagulation. The blood clot was removed and the slide was carefully drained with normal saline. A monolayer of polymorphonuclear leukocytes was inoculated with predetermined concentrations of test extracts for 15 min at 37 °C. The PMNs were covered with *Candida albicans* suspension for 1 hour. The slide was drained, fixed with methanol and stained with Giemsa stain [23]. The average number of PMN phagocytized Candida cells on the slide was determined microscopically per 100 granulocytes using standard morphological

criteria[24]. This number was the percentage of phagocytosis (PP) and was compared with PP of control. The stimulation of the phagocytic activity of PMNs cells was calculated using the following equation: Stimulation (%) = [(PP of test – PP of control)/PP of control] × 100

In vivo phagocytic activity

Experimental animals

The female Balb/c mice will be used in this study. Its weight should fall in an interval within ± 20 % of the mean initial weight of any previously dosed animals. The temperature in the experimental animal room should be 22 °C (± 3 °C). Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. For feeding, conventional rodent laboratory diets may be used with an unlimited supply of drinking water. The experiments will be performed according to the Animal Ethical Committee guidelines.

Carbon clearance test

The phagocytic activity of the reticuloendothelial systems was evaluated by a carbon clearance test [25] and the carbon clearance rate was measured by the method of Biozzi *et al.* (1955) [26]. Animals were randomized into four groups containing four mice each; namely G0, GI, GII and GIII. The treatment consisted of an intraperitoneal injection of the test substance. Animals in the control group (G0) were treated with 0.9 % NaCl (0.5 mL/mouse), and those in groups GI, GII and GIII were administered with AIRE at concentrations of 50, 100 and 500 mg/kg, respectively. After 48 hours of i.p. injection, the mice were administered with a carbon ink suspension at a dose of 0.1 mL/10 g in the tail vein; the mixture consisted of 3 mL of black carbon ink, 4 mL of saline and 4 mL of 3 % gelatin. Blood samples (25 µL) were then removed from the retro-orbital plexus before injection (0 min) and 10 minutes after the injection of the colloidal carbon ink via the heparin glass capillaries. The sample was then collected blood lysed in 0.1 % sodium carbonate solution (Na_2CO_3, 4 mL) and the optical density of the resulting solution was measured at 675 nm using a spectrophotometer. Phagocytic activity is expressed by the phagocytic index (K), which measures the entire function of the reticuloendothelial system in contact with circulating blood. The clearance rate is expressed as the half-life of carbon in the blood ($t_{1/2}$, min). These parameters are calculated using the following formulas [4, 25].

$$K = (\text{Ln } OD_1 - \text{Ln } OD_2)/(t_2 - t_1),\ t_{1/2} = 0.693/K$$

Where OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively.

Glutathione assay (GSH)

The animals were sacrificed and the liver and spleen dissected and weighed immediately in the wet state.

Preparation of the homogenate

The weight of 0.5 g of the liver was homogenized in 2 mL of TBS (Tris50 mM, NaCl 150 mM, pH 7.4). Then the homogenates were centrifuged at 9000 g for15 min at 4°C after that the supernatant was used for determination of glutathione reduced (GSH).

Method

The glutathione reduced content in the liver was measured spectrophotometrically by using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as a coloring reagent, following the method of Weckbeker *et al.* [27].

Statistical Analysis

All the results were expressed as the Mean ± Standard Error. The statistical analysis and the diagrams were performed using SPSS Statistic 17.0 and Graph Pad Prism 5 software. Differences between the means were statistically compared by one-way ANOVA via Tukey's Multiple Comparison Test. The values were considered significantly different when *P<0.05, **P< 0.01 and ***P<0.001.

RESULTS AND DISCUSSION

Extraction

The results obtained from extraction are reported in table 1. These results show that, the extraction yields obtained are ranged between 4.60 and 14.23 %, and *Allexis batangae* infused roots extract (AIRE) contains chemical constituents like flavonoids, phenols, tannins, triterpenes, anthocyanides, alkaloids, saponins, sugar and coumarins. Four compounds have been isolated from this extract 4,4''-dimethoxylophirone A, 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one, cornicula tusine and jaceosidine.

Characterization of Compounds Isolated from *Allexis batangae*

The structures of the isolated compounds were determined by spectroscopic analysis, especially, ^1H and ^{13}C NMR spectra in conjunction with 2D experiments, COSY, HSQC, HMBC and direct comparison with reference data from available literature [28-31]. The structures of all the isolated compounds are shown in Figure 1.

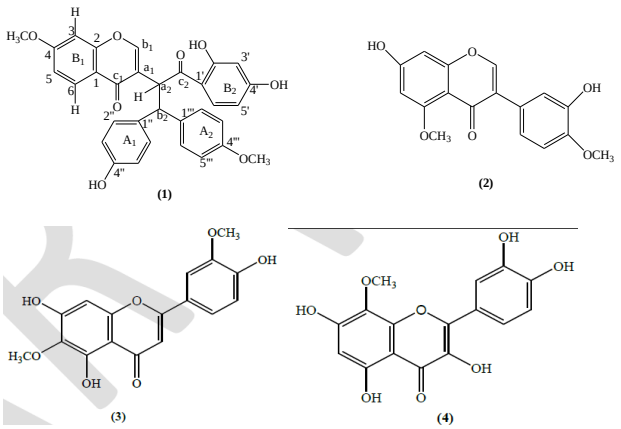

Figure 1: Structure of compounds **1** to **4** isolated from *Allexis batangae.*

Table 1: Extraction yield and chemical constituents of *Allexis batangae* infused roots extract (AIRE).

Extraction yield		
AIRE/Compounds	**Weight (mg)**	**Yield (%)**
AIRE		
4,4''-dimethoxylophirone A (1)	12.6	0.0126
7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one (2)	10.9	0.0109
Jaceosidine (3)	16.3	0.0163
Corniculatusine (4)	11.8	0.0118
Phytochemical screening on AIRE		
Phytomolecules	**Test procedures**	**Results**
Phenols		+ve
Flavonoids		+ve
Tannins	Ferric chloride test	+ve
Sterols	Salkowaski test	+ve
Triterpenoids	Salkowaski test	+ve
Anthocyanides		+ve
Alkaloids	Wagner's test	-ve
Saponins	Foam test	+ve
Reducing sugar	Fehling's test	+ve
Anthraquinones	Ammonia test 25 %	-ve
Coumarins	UV test	-ve
Proteins/Amino acids	Biuret test	+ve

+ve = Positive; -ve = Negative; AIRE = *Allexis batangae* infused roots extract.

The spectroscopic data of the different compounds isolated from *Allexis batangae* are shown in Tables 2, 3, 4 and 5.

Table 2: Spectroscopic data of 4,4''-dimethoxylophirone A **(1)** [28]

Position	δ_C mult	δ_H m, *J* (Hz)	HMBC
B1-1	112.87	-	-
2	158.0	-	-
3	102.3	6.72 (d; 2.5)	C-1; C-5
4	164.1	-	-
5	117.7	6.86 (dd; 2.5; 9)	C-1; C-3
6	127.6	7.94 (d; 9)	C-2; C-4; c1
c1	173.2	-	-
a1	121.5	-	-
b1	157.0	8.14(s)	C-2; c1; a1; a2
B2-1'	118.1	-	-
2'	165.5	-	-
3'	102.3	6,14 (d; 2)	C-1'; C-2'; C-4'; C-5'
4'	164.4	-	-
5'	103.2	6,38 (dd; 2; 9)	C-1'; C-3'
6'	134.0	6.17 (d; 9)	
c2	206.4	-	-
a2	44.4	6.11 (d; 12.2)	b1; b2; c2; a1; C-1''; C-1'''
b2	53.4	4.70 (d; 12.2)	C-1''; C-2''; C-1'''; C-2'''; c2
A1-1''	134.3	-	-
2''	129.0	7.27 (d; 8.5)	C-3'''; C-4''; b2
3''	114.7	6.63 (d; 8.5)	C-1''; C''-4''
4''	156.6	-	-
5''	114.6	6.63 (d; 8.5)	C-1''; C-3''; C'-4''
6''	128.0	7.29 (d; 8.5)	C-2''; C-4''; b2
A2-1'''	134.5	-	-
2'''	131.3	7.32 (d; 8.5)	C-3'''; C-4'''; b2
3'''	120.0	6.60 (d; 8.5)	C-1'''; C-4'''
4'''	158.6	-	-
5'''	117.9	6.60 (d; 8.5)	C-1'''; C-3'''; C-4'''
6'''	130.0	6.65 (d; 8.5)	C-2'''; C-4'''; b2
4'''-OCH_3	53.6	3.67 (s)	-

4-OCH_3	53.9	3.71	-
OH	-	-	-
OH	-	-	-

Table 3: Spectroscopic data of 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one **(2)** [29].

Position	^{13}C ppm	^{1}H ppm	HMBC
2	148,8	8,19 s	C-3 ; C-4 ; C-1’
3	125,1		
4	182,6		
4a	106,7		
5-OCH_3	164,9		
6	100,9	6,42 s	C-7 ; C-5 ; C-4a
7-OH	163,8		
8	95,5	6,28 s	C-7 ; C-6 ; C-8a ;
8a	160,0		
2’	148,8	6,93 d (1H, *J*=1,9)	
3’-OH	146,6		
4’-OCH_3	149,0		
5’	146,6	6,88 d (1H, *J*=7,9)	
6’	115,4	7,5 dd (1H, *J*=8,2-1,9)	
OCH_3	55,1	3,9 s (3H)	C-5’ ; C-4’ ; C-3’
OCH_3	55,0	3,91 s (3H)	C-5 ; C-4a ; C-6

Table 4: Data of Jaceosidin or 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (3)[30].

Chemicals shifts (ppm)	integration	Multiplicity (*J* Hz)	Attribution
12,85	1H	S	OH in C-5
7,45	1H	dd (8,4;2,1)	H-6’
7,32	1H	d (2,1)	H-2’
6,98	1H	d (8,4)	H-5’
6,56	1H	s	H-3
6,54	1H	s	H-8
3,85	3H	s	OCH3
3,80	3H	s	OCH3

Table 5: Spectroscopic data of Corniculatusine or 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-methoxy-4H-1-Benzopyran-4-one **(4)** [31].

Chemicals shifts (ppm)	integration	Multiplicity (*J* Hz)	Attribution
7.82	1H	S	H-2’
7.70	1H	d (8; 4)	H-6’
6.99	1H	d (8; 4)	H-5’
6.60	1H	S	H-6
3.87	3H	S	O-CH_3

Phagocytic activity results

In vitro phagocytic activity of polymorphonuclear (PMNs) cells results

The capacity of *Allexis batangae* extracts to modulate the phagocytic activity of human PMNs cells was tested against *Candida albicans* yeasts. The diagrams in figure 2 illustrate the phagocytosis and stimulation percentages obtained from substances tested. These graphs show that the stimulation of phagocytic activity was observed with *Allexis batangae* infused barks extract (AIRE), 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one (2) and jaceosidineor 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (3). The percentage of stimulation is ranged between 4.25 and 42.55 % with AIRE, 14.89 and 46.80 % with 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one (2), and between 4.25 and 59.57 % with jaceosidineor 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (3). Broth corniculatusine or 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-methoxy-4H-1-Benzopyran-4-one (4) and 4,4’’-dimethoxylophirone A (1) show stimulation of the phagocytic activity at lower concentrations and suppression at higher concentrations.

The results obtained show that stimulation of the phagocytic activity of PMNs cells have been observed at the concentrations ranged between 5 µg/mL (4.25 %) and 320 µg/mL (10.63 %); with the maximum of stimulation at 20 µg/mL (42.55 %). However, suppression of the phagocytosis at the concentration of 640 µg/mL (-4.25 %).

Carbon clearance test

The ability of *Allexis batangae* infused roots extract (AIRE) to enhance phagocytic activity was tested on Wistar rats by carbon clearance test. The figure 3 below shows that the mean for the phagocytic index (K) obtained from animal groups treated with AIRE (G1, G2, G3), native group (N) and control group (G0) is ranged between 0.020 ± 0.004 and 0.074 ± 0.006. Compared to control group G0 (K = 0.021 ± 0.006), the phagocytic index (K) was significantly increased with groups G1 (K = 0.040 ± 0.004, P < 0.01), G2 (K = 0.071 ± 0.008, P < 0.001) and G3 (K = 0.074 ± 0.006, P < 0.001). After 48 hr of intra peritoneal injection [Figure 4], the clearance rate of carbon was decreased to 34.890 ± 11.310 (control group G0) at 9.316 ± 0.821 min (group G3). The half life of colloidal carbon was significantly different in test groups G1 (P < 0.01), G2 (P < 0.001) and G3 (P < 0.001), compared to control group G0. The results obtained from the evaluation of the glutathione reduced content in the animal's liver [Figure 5] shows that the glutathione values are ranged between 2.100 ± 0.311 and 0.730 ± 0.107 nmol/mg of protein. There was a significant difference between control group G0 and test groups G1 (P < 0.01), G2 (P < 0.01) and G3 (P < 0.001). The difference was also observed between test groups G1 and G3 (P < 0.05). These results show that AIRE liberates the glutathione particles from liver and enhance the phagocytic activity by stimulating the reticuloendothelial system.

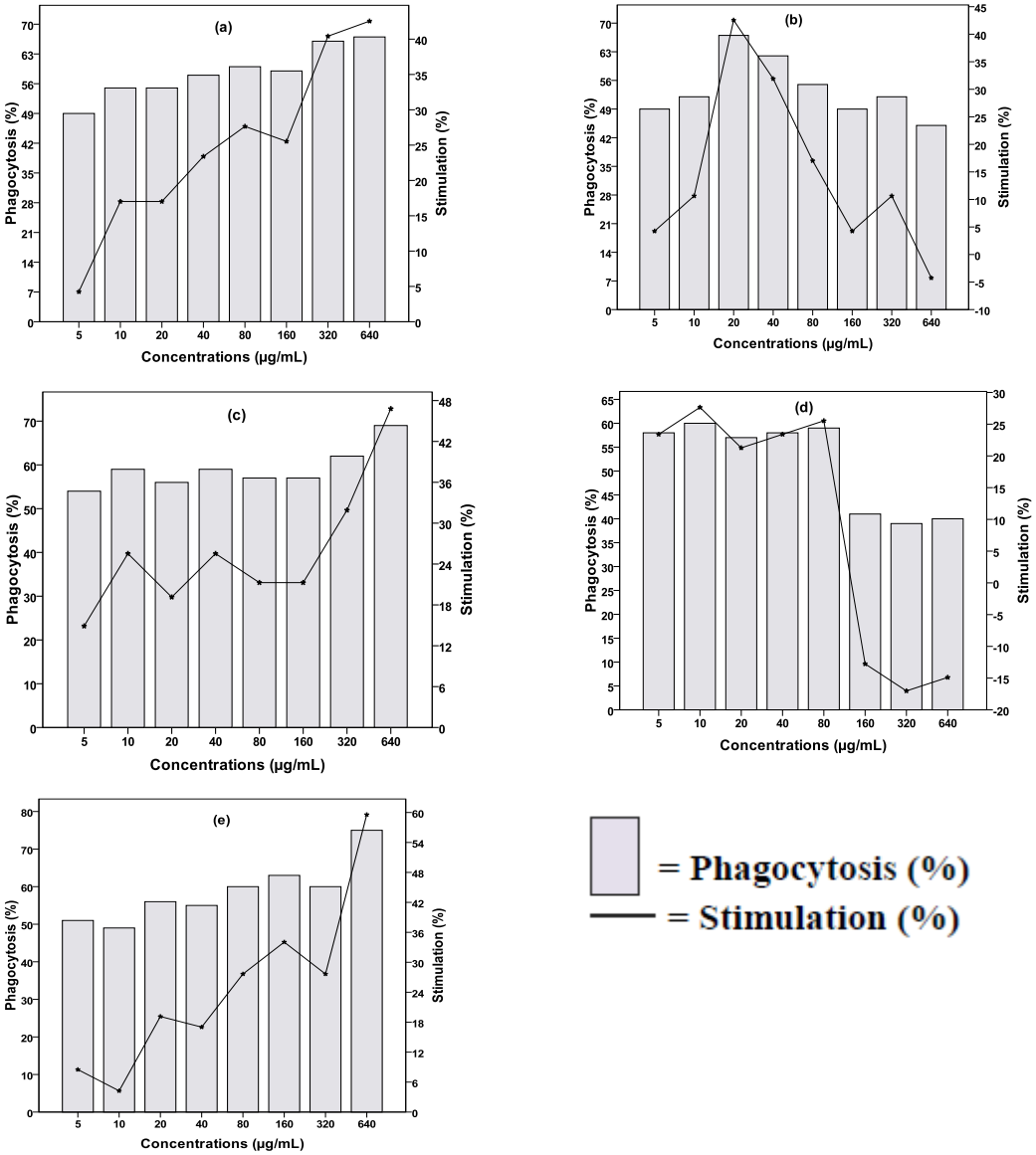

Figure 2: *Effects of Allexis batangae infused roots extract (a), 4,4''-dimethoxylophirone A (1); (b), **7**-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one (2) ; (c), jaceosidine or 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (3); (d) and corniculatusine or 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-methoxy-4H-1-Benzopyran-4-one (4); (e) on human neutrophils.*

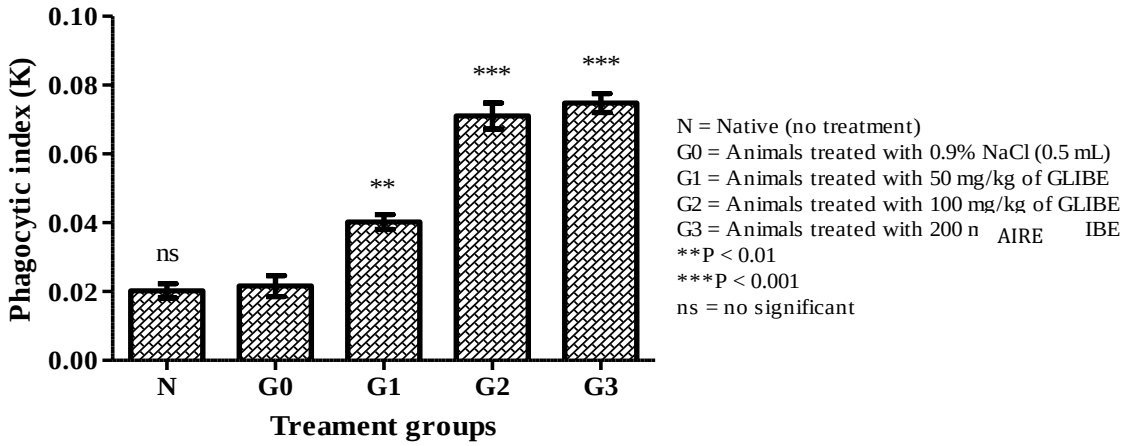

Figure 3: Effect of *Allexis batangae* infused roots extract on phagocytic index (K).

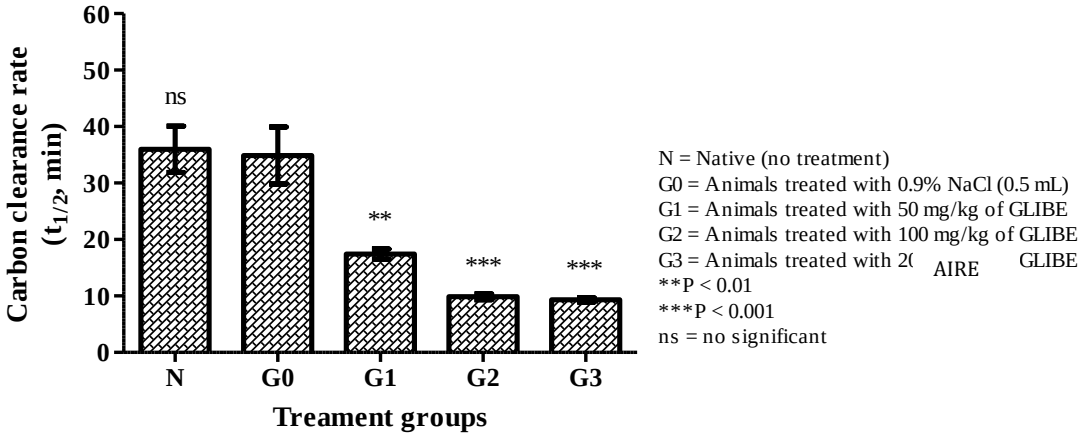

Figure 4: Effect of *Allexis batangae* infused roots extract on carbon clearance rate.

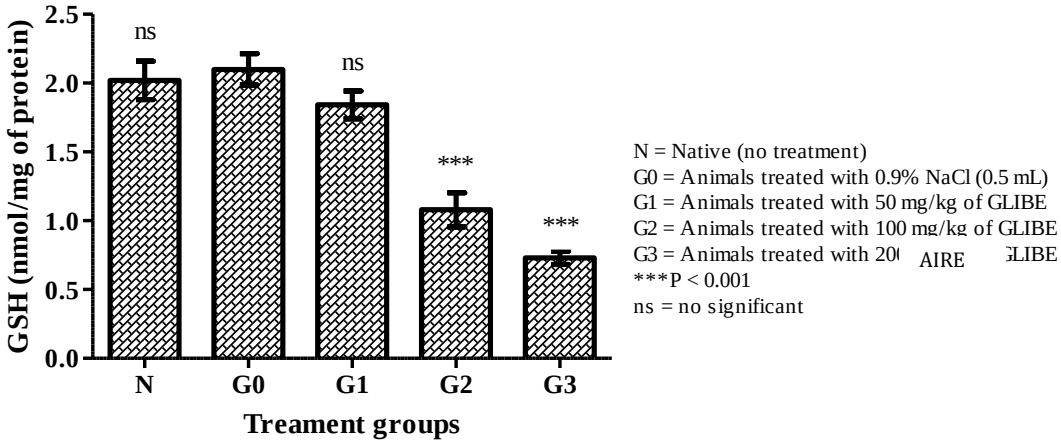

Figure 5: Effect of AIRE on glutathione reduced (GSH).

DISCUSSION

Active phagocytosis is the major defense mechanism against infection [32]. It is one of the most important host defense mechanism against invading microorganisms. Among phagocyte cells, there are polymorphonuclear neutrophils (PMNs) cells and macrophages. PMNs cells are the most abundant [33]. They are predominantly important in the removal of invading organisms from the body. They engulf these foreign bodies and degrade them using their powerful enzymes [10]. Macrophages are mononuclear phagocytes that contribute to development and homeostasis of immune response. They are highly phagocytic and, in this capacity, have long been considered to be essential immune effector cells [34,35]. The clearance rate of granular foreign bodies from circulation reflects the phagocytic function of macrophages [32]. The immune system

dysfunction is responsible for various diseases like allergy, asthma, arthritis and cancer. So, modulation of immune responses is much required for controlling the various diseases [10]. This study was carried out in order to evaluate the ability of *Allexis batangae* extracts to modulate phagocytic activity of human PMNs cells and macrophages.

Firstly, the phagocytic activity modulation of human PMNs cells by AIRE and flavonoids isolated was tested against *Candida albicans* yeasts. Stimulation of the phagocytic activity of human PMNs cells was observed with AIRE, 4,4''-dimethoxylophirone A (1) and jaceosidineor 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (3) at the concentrations tested. The stimulation percentage ranged between 4.25 and 42.55 % with AIRE, 14.89 and 46.80 % with (b), and between 4.25 and 59.57 % with (c). These compounds could be the potential agents for development of new drugs against immunosupression. Stimulation or suppression of the immune response may help in maintaining a disease-free state [12,36].

In this study, we also tested the capacity of AIRE to enhance activity of the reticuloendothelial system in Wistar rats. Measurement of the activity of the reticuloendothelial system depends upon estimation of the rate of clearance from the blood of foreign materials, such as colloidal carbon [4,37]. The carbon clearance test was conducted to establish phagocytic activity of reticuloendothelial system after treatment of animals with increasing doses of AIRE. The phagocytic index obtained from animal groups ranged between 0.020 ± 0.004 and 0.074 ± 0.006. Compared to control group, the phagocytic index was significantly increased in animals treated with AIRE ($P < 0.01$, $P < 0.001$). After 48 hr of i.p. injection, the clearance rate of carbon significantly decreased to 34.890 ± 11.310 at 9.316 ± 0.821 min. This reflects the enhancement of the phagocytic activity of mononuclear macrophage and non-specific immunity, which includes opsonisation of the foreign particulate matter with antibodies and complement C3b, leading to a more rapid clearance of foreign particulate matter from the blood [38]. In this *in vivo* assay, macrophages probably secrete a number of cytokines which in turn stimulate other immunocyte [22]. The results also show that the half-life of colloidal carbon was significant decreased in test animals compared to control group ($P < 0.001$); this means that AIRE enhance the phagocytic activity by stimulating the reticuloendothelial system. Moreover, glutathione reduced content in the animal's liver was significantly decreased in test animals. This shows that AIRE liberates the glutathione particles from animal's liver. Glutathione is a major antioxidant and a vital component of host defenses. In addition to protecting against free radical injury, it is important in the activation of lymphocytes, critical for the function of natural killer cells and lymphocyte-mediated cytotoxicity, and may have a role in the protection of neutrophils and macrophages against oxidative damage [25, 39]. The immunostimulant activity of AIRE may be attributed to his phytoconstituents.

In fact, the phytochemical screening realized on AIRE showed the relative presence of secondary metabolites among which are flavonoids, phenols, tannins, triterpenes, anthocyanides, alkaloids, saponins, sugar and coumarins. Most of these metabolites have already been cited in the literature for their immunomodulatory activities. For example, flavonoids like flovonols have been reported to stimulate human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T cells, cytokines, interleukin 2, gama-interferon and macrophages [40]. Furthermore, many bioactive compounds isolated from plants such as syringing, curcumin, flavopiridol, combretastatin and lycopene are mentioned in literature for their immunomodulatory effects [41,42].

Immunomodulation is the change in the body's immune system caused by the agents that activate or suppress its function[43]. The results obtained from this study indicate that infused roots extract and flavonoids from *Allexis batangae* modulate phagocytic activity and could be used as potential immunomodulatory agents.

CONCLUSION

The goal of the present study was to evaluate the immunomodulatory effects of infused roots extract and flavonoids from *Allexis batangae*, a plant of the Cameroonian pharmacopeia. The results obtained from this work support the use of extracts from this plant like potential source of new drugs to fight against immunological dysfunctions. *Allexis batangae* infused roots extract and flavonoids isolated have shown significant effects on non-specific immune response by enhancing the phagocytic activity. Further detailed studies will be helpful in elucidating the mechanism of immune-modulation by this plant. This study provides an agreement about the use of medicinal plants for the treatment of immune-related diseases.

Abbreviations Used: CC: Colum Chromatography; Cc: Cytotoxic concentration; PMNs: Polymorphonuclear; HMBC: Heteronuclear Multiple Bonding Connectivity; P: Picrorhizakurroa; HSQC: Heteronuclear Single Quantum Connectivity; COSY: Correlated Spectroscopy; HMBC: Heteronuclear Multiple Bonding Connectivity; RA: Rheumatoid Arthritis; TLC: Thin layer chromatography; UV: Ultraviolet; PI: Phagocytic index; i.p.: Intra-peritoneal; GSH: Glutathione reduced; DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid); G: Group; PP: Percentage of phagocytosis; K: Phagocytic index; ANOVA: Analysis of variance.

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Errata, dated Nov 01, 2020: Corrected Figure 1.