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Effects on the Phagocytosis Modulation of Stems Extract and Triterpenes from *Gouania longipetala* (Hemsl.), A Plant of The Cameroonian Pharmacopeia

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ABSTRACT

Immunomodulatory therapy is now being recognized as an alternative to conventional chemotherapy for a variety of disease conditions, involving the impaired immune response of the host. The goal of this study is to valorize *Gouania longipetala* (Hemsl.) like potential source of drugs against immune system dysfunction. This medicinal plant is commonly used in the Cameroonian pharmacopeia to cure a lot of diseases including inflammatory diseases. In this work, we evaluated the immunomodulation properties of infused stems extract (GLE) and triterpenes from *G. longipetala* (Hemsl.), with respect to the phagocytosis. GLE was obtained by infusion of dry bark powder in water and the triterpenes were isolated after fractionation of GLE in solvent systems following by column chromatography. The structure elucidation of isolated compounds was based on analysis of spectroscopic data including 1D- and 2D-NMR. The immunomodulation activity was evaluated by two methods. Firstly, the capacity of GLE and triterpenes to modulate phagocytic activity of polymorphonuclear (PMNs) cells was assessed *in vitro* against *Candida albicans*. Secondly, the ability of GLE to modulate reticuloendothelial system was investigated *in vivo* on wistar rats by carbon clearance test. Two triterpenes were isolated, alphitolic and epigouanic acids. The stimulation percentage of PMNs by plant extracts was ranged between 4.25 and 42.55% with GLE and between 14.89 and 46.80% with alphitolic acid. Epigouanic acid show biphasic activity profile, with stimulation of phagocytic activity of PMNs cells at lower concentrations and suppression effects at higher concentrations. *In vivo* test showed that GLE liberates the glutathione particles from animal's liver and enhance the phagocytic activity by stimulating the reticuloendothelial system. The phagocytic index obtained was ranged between 0.020 ± 0.004 and 0.074 ± 0.006. The results obtained from this study show that extract from *G. longipetala* (Hemsl.) modulate phagocytic activity and would be therefore the potential sources of drugs against immunological disorders.

1. Introduction

The immune system is designed to protect the host from invading pathogen and to eliminate disease [1]. It is involved in the etiology, as well as pathophysiologic mechanisms of many infectious diseases. Susceptibility to microbial, allergic and other disorders is higher in immunodeficiency state. In such conditions, the immune system's ability to fight infectious disease is compromised or entirely absent [2]. Immunomodulatory therapy is now being recognized as an alternative to conventional chemotherapy for a variety of disease conditions, involving the impaired immune response of the host [3,4]. The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and lymphocytes, and also to the production of various effectors molecules generated by activated cells. It is expected that theses nonspecific effects give protection against pathogens and constitute an alternative to conventional chemotherapy [5,1]. Immunomodulators have been known to support T-cell function, activate macrophages, granulocytes, complement and natural killer cells apart from affecting the production of various effectors molecules generated by activated cells [6].

Many of the presently available immunomodulators, such as levamisole, glucans, telerons, L-fucose, as well as *Cornybacterium parvum* bacterium, show one or other side effects that include fever, neutropenia, leucopenia and at times allergic reactions [7]. Hence, screening for new immunomodulators is an urgent need [8]. The search for more effective and safer agents exerting immunomodulatory activity is becoming a field of major interest all over the world [9]. Agents that regulate host defense mechanisms in the presence of an impaired or exaggerated immune responsiveness can provide supportive therapy to conventional chemotherapy [10,11].

Previous studies showed that some herbal medicine have immunomodulatory activities [12-14]. There are several herbs used in the indigenous systems of medicine that may modulate the body's immune system [15]. Herbal drugs are believed to enhance the natural resistance of the body against infection and their immunomodulatory activities have been reported in numerous plants extracts [16,17]. A variety of plant-derived materials such as lectins, peptides, phenolics, flavonoids, polysaccharides, tannins and saponins have been reported to modulate the immune system [15].

Several herbal preparations that can enhance the natural resistance of the body are extensively being used in the indigenous system of medicines [18,19]. Herbal preparations, originally used in the traditional systems of medicine, are now being investigated and effectively tried in a variety of pathophysiological states [20]. Medicinal plants are a rich source of substances which is the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer and complement functions [21]. They are traditionally used to treat immune disorders, such as inflammatory and autoimmune diseases. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases especially when host defense mechanism has to

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be acquired under the conditions of impaired immune responsiveness [22].

Gouania longipetala (Hemsl.) is a Rhamnaceae family plant commonly used in the Cameroonian pharmacopeia to cure a lot of diseases. Decoction of stems from this plant is traditionally used to treat stomach ache, gastro-intestinal infections, malaria, skin diseases, gynecological complaints and pregnancy problems. The leaves are used for the treatment of swellings, edema venomous stings, gout, febrifuges. It is also used as genital stimulants, laxatives and for treatment of venereal diseases. The leaf sap is used for eye treatments, as pain killers and for treating heart diseases [23]. Some previous studies concerning the pharmacological values of this plant have been published. For example, Ekuadzi et *al*. [24] in their research shown that the stem of this plant possessed antibacterial and anti-inflammatory activities. Ezeja et *al*. [23] evaluated toxicity profile of methanol leaf extract of the plant in rats.

To the best of our knowledge, no scientific study concerning the immunomodulator effects of *G. longipetala* (Hemsl.) extracts have been reported in the literature. This study was undertaken to evaluate the capacity of stems extract and triterpenes from this plant to modulate the phagocytic activity.

2. Experimental Methods

2.1 Plant Collection and Identification

The stems of *Gouania longipetala* (Hemsl.) was harvested in Banbjoun village (West-Cameroon region, 5° 22′ 31″ Nord, 10° 24′ 44″ Est, 1515 m of altitude) on September 15th 2017. The botanical identification was done at the National Herbarium of Cameroon where a specimen was deposed on No. 60787/HNC.

2.2 Preparation of Plant Infusion

After harvest, the stems collected were cute in scraps and air-dried at room temperature away from moisture. They dried stems were then crushed using electric grinder. A weighed quantity of 4 kg of powder was deducted and added to 20 L of boiling distilled water. The mixture was left to stand at room temperature. After 20 min, the solution was filtered through Whatman No 1 filter paper and the filtrate was lyophilized. Extraction was repeated three times. The extract obtained was weighted and stored at 4 °C until use. This extract has been codified GLE.

2.3 Phytochemical Screening

Qualitative phytochemical tests were done on GLE according the standard test procedures described in the literature by Sofowora [25] and Harbone [26].

2.4 Purification of Compounds and Structure Elucidation

A mass of 700 g of GLE was dissolved in 1 L of distilled water and then a liquid-liquid fractionation was carried out in 500 mL volumes of *n*-hexane, CH_2Cl_2 and *n*-butanol, respectively. The various fractions obtained were filtered and concentrated in a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany), and then oven-dried. A mass of 60 g of the *n*-hexane fraction was purified on silica gel column chromatography (4x150 cm, 250 g, 70-230 mesh) using *n*-Hexane/ethyl acetate (*n*-Hex/AcOEt) as elution system, with polarity increase of 10%. After elution, 158 sub-fractions of 100 mL were obtained.

Pre-coated silica gel 60 F254 thin layer chromatography plates (TLC, Merck, Germany) were used for monitoring fractions; spots were detected using UV light (254 and 365 nm) and sprayed with 30% sulphuric acid (H_2SO_4) followed by heating at 110 °C. Basing to their TLC profiles, these sub-fractions were regrouped in 6 fractions namely Sf1, Sf2, Sf3, Sf4, Sf5 and Sf6. The fractions Sf1 and Sf3 were further purified on CC using *n*-Hex/AcOEt elution system. After elution, 108 and 183 sub-fractions of 100 mL were obtained from Sf1 and Sf3, respectively. Based on their TLC profile, these sub-fractions were subsequently grouped into 4 fractions for Sf1 (Sf^1a, Sf^1b, Sf^1c and Sf^1d) and 6 fractions for Sf3 (Sf^3a, Sf^3b, Sf^3c, Sf^3d, Sf^3e and Sf^3f). The fractions Sf^1b and Sf^3d gave two compounds. The structure elucidation of these compounds was assessed by analysis of 1D (1H) and 2D (HSQC, HMBC) NMR spectra obtained at frequencies of 800 and 201 MHz, respectively.

2.5 Phagocytic Activity

2.5.1 In Vitro Phagocytic Activity of Polymorphonuclear (PMNs) Cells

The effect of *G. longipetala* extracts on immunomodulation of human polymorphonuclear cells was evaluated by the method described by Ponkshe and Madhavi [27], with little modifications. From a *Candida albicans* culture of 24 hours on Sabouraud Dextrose Agar plate, the inoculum was prepared by suspending a pure colony of yeast in 1 mL of human serum 1:4 in salt solution NaCl 0.9%. The concentration of cell suspension was adjusted at 10^8 UFC/mL by microscopy counting. Human blood (0.2 mL) was deposited on a sterile glass slide. Slides in triplicate were incubated at 35 °C for 25 min to allow clotting. The blood clot was removed and the slide drained carefully with normal saline. Monolayer of polymorphonuclear leucocytes was flooded with predetermined concentrations of the test extracts for 15 min at 37 °C. The PMNs were covered with *C. albicans* suspension for 1 hour. The slide was drained, fixed with methanol and stained with Giemsa stain [28]. The mean number of Candida cells phagocytosed by PMNs on the slide was determined microscopically for 100 granulocytes using standard morphological criteria [29]. 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:

$$\text{Stimulation (\%)} = [(\text{PP of test} - \text{PP of control})/\text{PP of control}] \times 100$$

2.5.2 In Vivo Phagocytic Activity

2.5.2.1 Experimental Animals

Wistar rats weighting 135 ± 10 g were selected for the experiment. These animals were raised in the Animal House of Department of Animal Biology of the University of Yaounde I where, they were maintained under standardized condition (12 h light/dark cycles, room temperature of 22 ± 3°C). They were provided with conventional rodent laboratory diets and free access to drinking water *ad-libitum*. Prior the experimental tests, an authorization was obtained from the Cameroon National Ethical Committee. The experiment was performed according to the Animal Ethical Committee guidelines.

2.5.2.2 Carbon Clearance Test

Phagocytic activity of reticuloendothelial systems was assayed by carbon clearance test according the protocol described by Kehili et *al.* [30]. Animals were randomized into five groups containing 4 rats each; namely N, G0, G1, G2, G3 and G4. The treatment consisted to intra-peritoneal (i.p.) injection of the tested substance. The animals in group G0 (Control) were treated with 0.9% NaCl (0.5 mL/mouse), and those in groups G1, G2, G3 and G4 were administered with GLE at the concentrations of 25, 50, 100 and 200 mg/kg, respectively. After 48 h of i.p. injection, animals were administered with carbon ink suspension at a dose of 0.1 mL/10 g through the tail vein; the mixture consisted of 3 mL black carbon ink, 4 mL of saline and 4 mL of gelatin 3%. Blood samples (25 µL) were withdrawn from the retro-orbital plexus before injection and 10 minutes after injection of colloidal carbon ink capillary tube. The blood collected was then lysed in 0.1% sodium carbonate solution (Na_2CO_3, 4 mL) and the optical density of the solution obtained was measured at 675 nm. Animals in group N (native group) were no treated with plant extract and no administrated with carbon ink suspension. The phagocytic activity was expressed by the phagocytic index (K) which measures all the reticuloendothelial system function in the contact with the circulating blood. The clearance rate was also determinate and expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min). These parameters are calculated using the following formulas [4]:

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

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

2.5.2.3 Glutathione Assay (GSH)

The animals were sacrificed and after dissection, the liver was deducted**.** The weight of 1 g of the liver was homogenized in 2 mL of trichloroacetic acid TCA 5%. Then the homogenate was centrifuged at 2000 rpm for 20 min at 4 °C. The supernatant was deducted and used for the determination of glutathione reduced (GSH); following the method described by Ellman [31]. Briefly, 50 µL of the supernatant was diluted in 950 µL of phosphate buffer (0.1 M; pH = 8) and then 20 µL of 5,5′-dithiobis-2-nitrobenzoic acid (0.01 M) was added to 3 mL of the obtained dilution. The optical density of this last solution was read at 412 nm against a blank prepared in the same condition with TCA 5%. The concentration of GSH was expressed in mmol of GSH/g of liver using a standard curve realized with glutathione in same conditions.

2.6 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 GraphPad Prism 5 software. Differences between the means were

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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.

3. Results and Discussion

3.1 Extraction

The extraction yield of the infused *G. longipetala* stems extract (GLE) was 32.47%. The results obtained from phytochemical screening (Table I) showed that GLE contains metabolites groups like flavonoids, phenols, tannins, triterpenes, anthraquinones, anthocyanides, alkaloids, lignins, saponins, sugar, sterols coumarins and proteins. After successive fractionation of this extract on silica gel column chromatography, two triterpenes have been isolated, alphitolic and epigouanic acids.

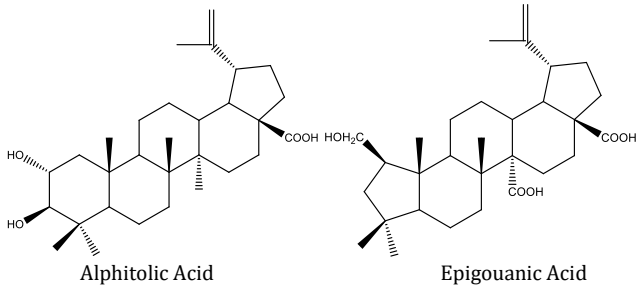
Table 1 Chemical constituents of the infused *G. longipetala* stems extract (GLE)

Phytomolecules	Test procedures	Results
Phenols	Ferric chloride test	+ve
Flavonoids	NaOH test	+ve
Tannins	Ferric chloride test	+ve
Sterols	Liebermann-Burchard	+ve
Triterpenoids	test	+ve
Lignins	Labat test	-ve
Alkaloids	Mayer test	+ve
Saponins	Foam test	+ve
Reducing sugar	Fehling's test	+ve
Anthocyanines	H_2SO_4 test	-ve

+ve, Positive; -ve, Negative; GLE, *G. longipetala* infused stems extract

3.2 Characterization of Compounds Isolated

The structure elucidation of the compounds isolated was based on analysis of spectroscopic data obtained from 1D (1H) and 2D (HSQC, HMBC) NMR spectra, at frequencies of 800 and 201 MHz, respectively. These data were compared to those mentioned in the literature by other authors concerning these compounds. The ^{1}H-NMR and ^{13}C NMR data obtained from each compound are mentioned below. The ChemDraw Pro 8.0 Software for windows was used to draw the chemical structures of compounds isolated (Fig. 1).



Alphitolic Acid Epigouanic Acid

Fig. 1 Chemical structure of alphitolic acid and epigouanic acid

3.2.1 Alphitolic Acid

^{1}H-NMR (800 MHz, $CDCl_3$) δ (ppm): 0.80, 0.90, 0.94, 0.98 and 1.01 (5s, 15H, all tertiary –CH_3), 1.41 (m, 2H, H-21), 1.34 (m, 2H, H-16), 1.43 (m, 2H, H-20), 1.53 (m, 4H, H-18, H-19 and H-15), 2.20 (m, 3H, H-1 and H-9), 2.28 (m,2H, H-14), 2.98 (t, 2H, J = 7 Hz, H-2), 3.51 (s, 1H, H-7), 4.52 (s, 2H, H-11), 4.74 (s, 2H, H-12).

^{13}C NMR (CD_3OD, 201 MHz) δ (ppm): 39.19 (C-1), 69.26 (C-2), 83.94 (C-3), 39.19 (C-4), 56.24 (C-5), 18.28 (C-6), 34.24 (C-7), 40.80 (C-8), 50.49 (C-9), 38.33 (C-10), 20.98 (C-11), 25.40 (C-12), 38.60 (C-13), 42.51 (C-14), 32.13 (C-15), 34.24 (C-16), 56.24 (C-17), 46.88 (C-18), 49.28 (C-19), 150.25 (C-20), 29.65 (C-21), 30.56 (C-22), 28.47 (C-23), 17.38 (C-24), 16.49 (C-25), 16.08 (C-26), 14.67 (C-27), 179.51 (C-28), 109.77 (C-29), 19.36 (C-30).

3.2.2 Epigouanic Acid

^{1}H-NMR (800 MHz, CD_3OD): δ = H-1 (d, J = 8Hz ; 5.4); .H-3 (d, J = 8Hz ; 5.96); H-5 (dd, J = 4.66, 9.23 Hz; 1.14); H-9 (d, J = 11.41 Hz; 1.88); H-13 (m, 2.41); H-18 (m, 1.69); H-19 (m, 3.3); H 23 (s, 1.04); H-24 (s, 0.91); H-25 (s, 0.98); H-26 (s, 1.07); Ha-29 (s, 4.58); Hb-29 (s, 4.52); H-30 (s, 1.70).

^{13}C NMR (CD_3OD, 201 MHz) δ (ppm): 44.89 (C-1), 63.35 (C-2), 42.52 (C-3), 38.79 (C-4), 58.87 (C-5), 19.23 (C-6), 35.53 (C-7), 41.82 (C-8), 52.58 (C-9), 48.78 (C-10), 24.21 (C-11), 27.02 (C-12), 41.26 (C-13), 61.09 (C-14), 29.21 (C-15), 38.25 (C-16), 57.29 (C-17), 52.98 (C-18), 48.68 (C-19), 151.54 (C-20), 31.55 (C-21), 38.55 (C-22), 34.20 (C-23), 27.06 (C-24), 19.65 (C-25), 18.31 (C-26), 180.14 (C-27), 179.26 (C-28), 110.56 (C-29), 20.40 (C-30).

3.3 Phagocytic Activity

3.3.1 In Vitro Phagocytic Activity of Polymorphonuclear (PMNs) Cells

The capacity of *G. longipetala* extracts to modulate the phagocytic activity of human PMNs cells was tested against *Candida albicans* yeasts. The diagrams in Fig. 2 illustrate the phagocytosis and stimulation percentages obtained from the tested substances. These graphs show that the stimulation of phagocytic activity was observed with *G. longipetala* infused stems extract (GLE) and alphitolic acid with percentages of stimulation is ranged between 4.25 and 42.55% for GLE and between 14.89 and 46.80% for alphitolic acid. Epigouanic acid shows a biphasic activity profile. From this compound, a stimulation of the phagocytic activity of PMNs cells have been observed at the concentrations ranged between 5 µg/mL (25.40 %) and 80 µg/mL (25.53 %), and a suppression activity has been observed between 160 µg/mL (- 12.76 %) and 640 µg/mL (- 14.89 %). At the concentrations tested, alphitolic acid exhibited the highest stimulatory activity at the concentration of 640 µg/mL (46.80%).

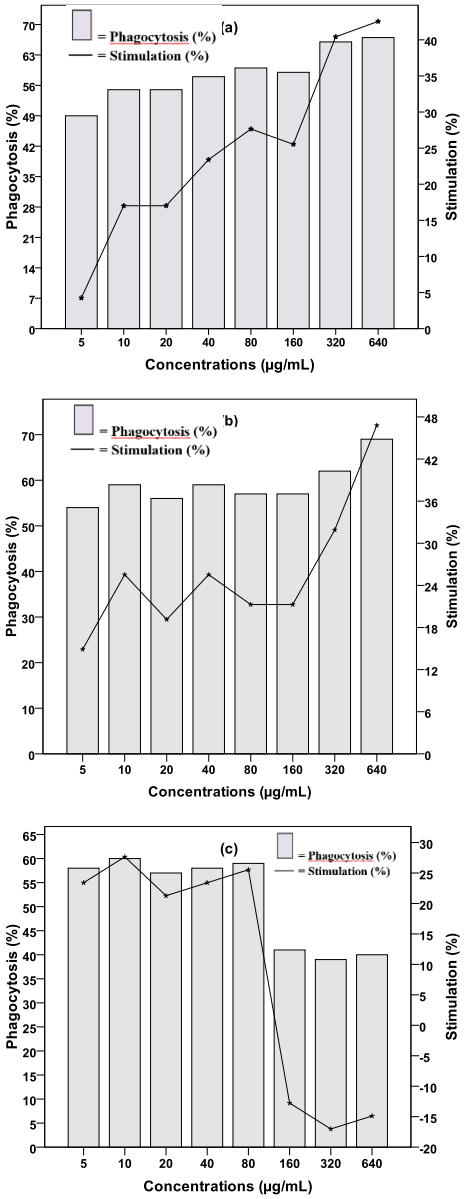


Fig. 2 Immunomodulator effects of *G. longipetala* infused stems extract (a), alphitolic acid (b) and epigouanic acid (c) on human polymorphonuclear cells

3.3.2 Carbon Clearance Test

The ability of infused *G. longipetala* stems extract (GLE) to enhance phagocytic activity was tested on Wistar rats by carbon clearance test. The Fig. 3 shows that the means for the phagocytic index (K) obtained from

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animal groups treated with GLE (G1, G2, G3, G4) and control group (G0) is ranged between 0.020 ± 0.004 and 0.074 ± 0.006. Compared to control group G0 (K = 0.020 ± 0.004), the phagocytic index (K) was significantly increase with groups G2 (K = 0.040 ± 0.004, P < 0.01), G3 (K = 0.071 ± 0.008, P < 0.001) and G4 (K = 0.068 ± 0.009, P < 0.001). After 48 h of intra peritoneal injection (Fig. 4), the clearance rate of carbon was decreased to 35.971 ± 9.127 (group G0) at 9.868 ± 1.167 min (group G3) and the decreasing become significant from the concentration of 50 mg/kg of GLE (group G2, P < 0.05). The half time of colloidal carbon was significantly different in test groups G2 ($t_{1/2}$ = 17.426 ± 1.967 min, P < 0.01), G3 ($t_{1/2}$ = 9.868 ± 1.167 min, P < 0.001) and G4 ($t_{1/2}$ = 10.230 ± 1.492 min, P < 0.001), compared to control group G0 ($t_{1/2}$ = 35.971 ± 9.127 min). The results obtained from the evaluation of the glutathione reduced content in the animal's liver (Fig. 5) show that the glutathione values are ranged between 2.100 ± 0.311 and 0.730 ± 0.107 mmol of GSH/g of liver. There was a significant difference between control group G0 and test groups G3 and G4 (P < 0.001). These results show that GLE liberates the glutathione particles from liver and enhance the phagocytic activity by stimulating the reticuloendothelial system.

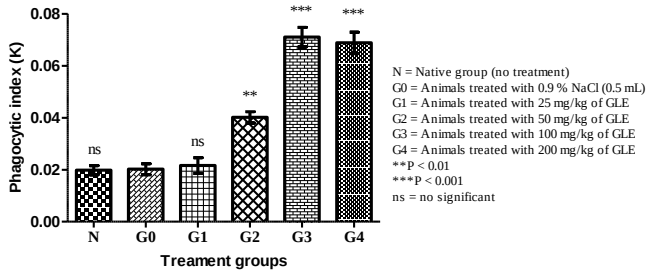

Fig. 3 Effect of *G. longipetala* infused stems extract (GLE) on phagocytic index (K)

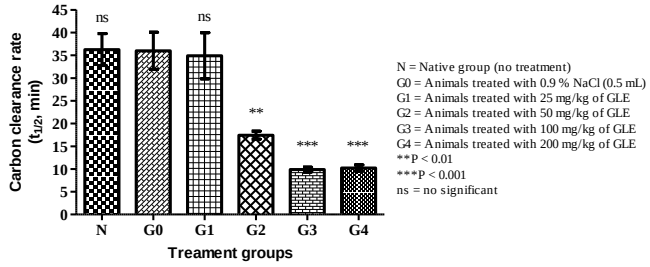

Fig. 4 Effect of *G. longipetala* infused stems extract (GLE) on carbon clearance rate

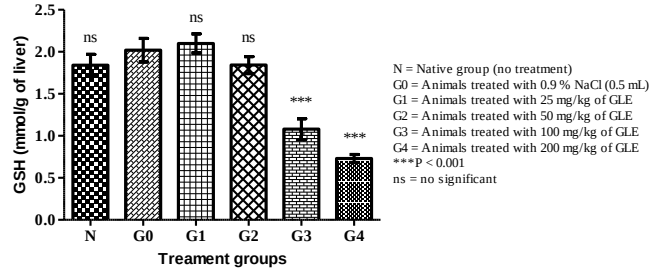

Fig. 5 Effect of *G. longipetala* infused stems extract (GLE) on glutathione reduced (GSH)

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 [9]. 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]. 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 too much required to controlling the various diseases [9]. This study was carried out in order to evaluate the ability of *G. longipetala* (Hemsl.) extracts to modulate phagocytic activity of human PMNs cells and macrophages.

Firstly, the phagocytic activity modulation of human PMNs cells by infused *G. longipetala* stems extract (GLE) and triterpenes isolated was tested against *Candida albicans* yeasts. Stimulation of the phagocytic activity of human PMNs cells was observed with GLE and alphitolic acid at the concentrations tested. The stimulation percentage is ranged between 4.25 and 42.55% with GLE and between 14.89 and 46.80% with alphitolic acid. These compounds could be the potential agents for development of new drugs against immunosupression. Epigouanic acid shows a biphasic activity profile with stimulation of phagocytic activity at lower concentrations and its suppression at higher concentrations. This suggests that this compound may act as cytotoxic agent at higher concentrations but can also act as immunostimulant when applied in small doses [27]. Stimulation or suppression of the immune response through may help in maintaining a disease-free state [10,11]. These compounds could be exploited in the development of new drugs with immunomodulatory activity.

In this study, we also tested the capacity of GLE 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 [35,4]. The carbon clearance test was conducted to establish phagocytic activity of reticuloendothelial system after treatment of animals with increasing doses of GLE. The phagocytic index obtained from animal groups is ranged between 0.020 ± 0.004 and 0.074 ± 0.006. Compared to control group, the phagocytic index was significantly increase in animals treated with GLE (P < 0.01, P < 0.001). After 48 h of i.p injection, the clearance rate of carbon was significantly decreased to 35.971 ± 9.127 at 9.868 ± 1.167 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 [36]. Macrophages probably secrete a number of cytokines which in turn stimulate other immunocyte [27]. The results also show that the half time of colloidal carbon was significant decreased in test animals compared to control group (P < 0.01, P < 0.001); this means that GLE enhance the phagocytic activity by stimulating the reticuloendothelial system. Moreover, glutathione reduced content in the animal's liver was significant decreased in test animals. This shows that GLE 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 [37,30]. The immunostimulant activity of GLE may be attributed to his phytoconstituents.

In fact, the phytochemical screening realized on GLE showed the relatively presence of secondary metabolites among which 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 [8]. Furthermore, many bioactive compounds isolated from plants such as syringing, curcumin, flavopiridol, combretastatin and lycopene are mentioned in literature for their immunomodulatory effects [38,39].

Immunomodulation is the changes in the body's immune system caused by the agents that activate or suppress its function [40]. The results obtained from this study indicate that infused stems extract and triterpenes from *Gouania longipetala* (Hemsl.) modulate phagocytic activity and could be used as potential immunomodulator agents.

4. Conclusion

In this study, we evaluated the immunomodulatory effects of infused stems extract and triterpenes from *Gouania longipetala* (Hemsl.), a plant of the Cameroonian pharmacopeia. The results obtained support the use of extracts from this plant like potential sources of new drugs to fight against immunological dysfunctions. *Gouania longipetala* infused stems extract and triterpenes 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.

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