

Full Length Research Paper

Immunomodulatory activities of polysaccharides isolated from plants used as antimalarial in Mali

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Medicinal plants used against malaria in Mali have previously been tested for their antiplasmodial activities using their organic solvent and water extracts. As the healers mainly use the water extracts for their treatments of malaria-patients, our aim was to study the water-soluble components from Malian plants used for treatment of malaria. *Argemone mexicana* (aerial parts), *Sarcocephalus latifolius* (root bark), *Vitex doniana* (leaves), and Malarial-5[®] (an improved traditional medicine (ITM) in tea) were the objects of our studies. Water extracts of these plants contained primarily polysaccharides. Due to this, the studies focused on the determination of the monosaccharide composition of the polymers present as well as assessing the immunomodulatory properties of the polysaccharide fractions isolated from these plants. Each plant material was extracted sequentially with dichloromethane, 80% ethanol and water at 100°C. The polysaccharides were obtained using gel filtration of the aqueous extracts and their monosaccharide compositions were determined using gas chromatography. Immunomodulatory effects were assessed using the complement fixation test and macrophage stimulation. All aqueous extracts from the four samples contained polysaccharides. The monosaccharide compositions vary between the plants. Arabinose, rhamnose, galactose, glucose and galacturonic acid were present in all samples, glucose being the main monomer. These polysaccharides showed complement fixing activity and induced nitrite oxide release from macrophages in a dose dependent manner. The polysaccharide fractions of *A. mexicana* (Am1) and *V. doniana* (Vd1) showed the most potent activities. These two fractions had an ICH₅₀ of 2.4 and 6.3 µg/mL respectively in the complement fixation assay. The same two fractions induced a dose dependent release of nitrite oxide from macrophages. The results demonstrated that antimalarial plants contain polysaccharides with immunomodulatory properties. This preliminary work constitutes a new approach of antimalarial studies.

Key words: Polysaccharides, immunomodulatory effects, antimalarial plants, Mali.

INTRODUCTION

In 2017, World Health Organization (WHO) estimated 219 million cases of malaria in 90 countries with 435,000

deaths worldwide. The African region registered 92% of the malaria cases and 93% of the deaths (WHO, 2017).

This infectious disease caused by parasites is transmitted to people through the bites of infected female anopheles mosquitoes (WHO, 2017). *Plasmodium falciparum* is mainly responsible for the enormous deaths (99%), rarely caused by other *Plasmodium* species (Belachew, 2018). Malaria is the primary cause of morbidity and mortality in Mali, particularly among children under the age of five (Anonymous, 2018). The entire population of Mali is at risk for malaria. The disease is endemic in the central and southern regions where more than 90 percent of the population lives, and it is also epidemic in the north (Anonymous, 2018).

In Mali, the current health system is decentralized, and is composed of three levels, which involves an integrated community case management package at the community level (Anonymous, 2018). Most of the population is still using traditional medicines for their primary health care. Plants have been used to treat malaria for thousands of years and are the source of the two main groups of modern antimalarial drugs (quinine and artemisinin derivatives) according to Willcox and Bodeker, (2004). RTS,S/AS01 (RTS,S) also known as Mosquirix® is an injectable vaccine that offers protection against malaria in young children, but it is unavailable for the population where malaria is endemic (Anonymous, 2018). The problems of increasing levels of artemisinin-resistant parasites encourage researchers for finding a new source of anti-parasitic drugs. Since developing countries have difficulties in affording and accessing effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment (Willcox and Bodeker, 2004). Therefore the exigent need of effective molecules remains a huge challenge for scientists. Most of the recent investigations on antimalarial plants have been focused on organic solvent extracts. But in Mali, the department of traditional medicine (DMT) in order to supersede its first Malian improved traditional medicine for malaria (Malarial-5®) has demonstrated the efficacy and safety of aqueous extracts through preclinical and clinical studies including *Sumafura Tiemoko Bengalyan* herbal tea based on *Argemone mexicana* that came out from a retrospective treatment-outcome study (Diallo et al., 2007; Willcox et al., 2007; Sanogo et al., 2008; Graz et al., 2010; Willcox et al., 2011). The phytochemical analysis and biological activities on *Sumafura Tiemoko Bengalyan* an herbal tea led to formulate syrups for an efficient utilisation and its standardization (Sanogo et al., 2012, 2014). This new antimalarial phytomedicine made by the department of traditional medicine of Mali retrieves its name from one traditional healer, *Tiemoko Bengaly*, who has participated in its development (Willcox, 2011). This author reported

that several alkaloids including berberine, protopine, and allocryptopine from *A. mexicana* exhibited *in vitro* antimalarial effect while animal studies suggest that the crude aqueous extract is not effective against *Plasmodium berghei*, and berberine also is not well absorbed orally (Willcox, 2011). Thus, some investigations were underway to identify which compounds are active in humans. Most of traditional healers in Mali are thinking that plants mainly should be taken as water extract. Therefore a new approach is urgently needed to find a product for prevention and treatment of malaria. Although parasites have their own ways to develop resistance against drugs, the immune system has naturally evolved to arm the host against pathogens, including parasites. Both innate and adaptive immune responses selectively recognize pathogens and help the host to get rid of many of them at first sight (Coban and Yamamoto, 2018). Due to this, our theory is that the healing effect observed with patients using aqueous plant extracts could partly be due to their stimulating activity of the immune system.

In Malian traditional medicine, water decoction is the most popular mode of preparation of plants remedies and polysaccharides isolated from those crude water extracts have shown effects related to the immune system through various *in vitro* and *in vivo* tests (Paulsen and Barsett, 2005). Diallo and coworkers showed that the pectic polysaccharides isolated from the leaves of *Trichilia emetica* (Meliaceae), a plant used in traditional medicine in Mali, activated the complement system and induced the proliferation of T and B-lymphocytes (Diallo et al., 2003). *Biophytum petersianum*, traditionally used in Mali for wound healing contains polysaccharides with complement fixing activity (Inngjerdigen et al., 2006; Inngjerdigen et al., 2008; Grønhaug et al., 2011). *Opilia celtidifolia* used traditionally against skin diseases and malaria is also known as appetizer plant in Mali. Polysaccharide fractions from that species exhibited complement fixation and macrophage stimulation activities (Diallo et al., 2003; Togola et al., 2008; Šutovská et al., 2009). Investigations on the roots of *Vernonia kotschyana* used to produce Gastro-sedal, an improved traditional medicine in Mali, revealed that its polysaccharides possessed a complement fixation activity (Nergard et al., 2005; Inngjerdigen et al., 2012). Recently, two other Malian medicinal plants, *Parkia biglobosa* and *Terminalia macroptera* were reported to contain polysaccharides having complement fixing and macrophage stimulating effects (Zou et al., 2014a, b). Often, pectic extracts prepared using hot water, were found to be active on the complement system. Aboughe-Angone et al. (2011) reported that plant water soluble

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compounds like pectic and hemicellulosic polysaccharides have immunomodulatory and mitogenic (proliferation of B-lymphocytes) properties. Plant polysaccharides can directly activate the immune function of macrophages, T or B lymphocytes, natural killer cells, and complement (Yu et al., 2017). Macrophages stimulated with lipopolysaccharides (LPS) or an immunomodulatory compound produce large amounts of free radicals such as nitric oxide (NO), which effectively suppressed the blood stage of the malarial parasite (Awasthi et al., 2003). Thus, the present study aimed to investigate the components and the immunomodulatory effects of polysaccharides from antimalarial plants used in Mali.

MATERIALS AND METHODS

Plant material

Three plants and one improved traditional medicine used frequently in Mali against malaria without prior knowledge on their immunomodulatory properties were selected for the present study. The plant materials were *A. mexicana* L., Papaveraceae (aerial parts), *Sarcocephalus latifolius* (Sm.) E.A. Bruce, Rubiaceae (root barks), *Vitex doniana* Sweet, Lamiaceae (leaves), and Malarial-5[®] (improved traditional medicine containing *Senna occidentalis* (L.) Link. (syn. *Cassia occidentalis* L.) (leaves), *Lippia chevalieri* Moldenke (leaves) and *Acmella oleracea* (L.) R.K.Jansen. (syn. *Spilanthes oleracea* L.) (flowers) presented as herbal tea. *A. mexicana*, *S. latifolius* and *V. doniana*, were bought at the market of Medine in Bamako, Mali, in 2012, identified by Professor Drissa Diallo, Department of Traditional Medicine, Bamako, Mali, and voucher specimen were deposited at the herbarium of the DMT (Voucher No 2948 / DMT, 2198 / DMT, 2008 / DMT respectively). The Plant List website (www.theplantlist.org) was accessed in February 2019 for correct Latin names of the plants. The plant materials were air dried at room temperature and pulverized into fine powder by a mechanical grinder. The tea form of Malarial-5[®], which is an improved traditional medicine of DMT, was provided by this institution. The powders and the herbal tea were used for the extraction.

Method of extraction

The powdered leaves, root bark, aerial parts and the formulated herbal tea (50 g of each) were extracted with dichloromethane using the Soxhlet system, followed by maceration in 80% ethanol in order to remove lipophilic compounds and colored materials. The residues were then extracted with water at 100 °C for 1 h, filtered through glass fiber filter, and concentrated at 40°C under vacuum with an evaporator. The concentrated solutions were frozen to give the crude water extracts called Vd, Sl, Am and Ma respectively for *V. doniana*, *S. latifolius*, *A. mexicana* and Malarial-5[®].

Fractionation of polysaccharides by chromatography on Biogel P6

The aqueous extracts (Vd, Sl, Am and Ma) were dissolved in distilled water, centrifuged, filtered through a Millipore filter (5 µm) and gel filtered on a Biogel P6 column (5 cm × 60 cm) using distilled water as the mobile phase. The fractions, from high to low molecular weights, were identified based on their elution profiles as tested by the phenol sulphuric acid method (Dubois et al., 1956).

Three fractions (1, 2 and 3) of Vd, Sl and Am; and two fractions (1 and 2) of Ma contained high molecular weight material. Each fraction was pooled, concentrated and freeze-dried to give polysaccharide fractions. The samples retained for further studies were called Vd1, Vd2, Vd3, Sl1, Sl2, Sl3, Am1, Am2, Am3, Ma1 and Ma2.

Determination of monosaccharide composition of the fractions

One milligram of the lyophilized polysaccharide of each sample was subjected to methanolysis for 24 h (80°C) using water free 3 M HCl in MeOH (Sigma–Aldrich) (Chambers and Clamp, 1971). Hundred microliters of mannitol (1 mg/mL) were added as an internal standard. After 24 h reaction time, the reagents were removed with nitrogen and the methyl-glycosides dried in vacuum over P₂O₅ for 1 h prior to their conversion into the corresponding trimethyl silylethers (TMS-derivates). The samples were analyzed by capillary gas chromatography (30 m × 0.32 mm, J and W Scientific Inc.) on a Carlo Erba 6000 Vega Series 2 gas chromatograph with an ICU 600 programmer (Chambers and Clamp, 1971; Barsett et al., 1992). The injector temperature was 250°C, the detector temperature 300°C and the column temperature was 140°C when injected, then increased with 1°C/mn to 170°C, followed by 6°C/mn to 248°C and then 30°C/mn to 300°C. Helium was used as carrier gas with a flow rate adjusted to a retention time of 33 min for the internal standard. Based on standards for all the monomers present, the monosaccharides were identified and quantified.

Immunomodulatory activities

The complement fixation test

The complement fixation test is based on inhibition of haemolysis of antibody sensitized sheep red blood cells (SRBC) by complement from human sera as described by Michaelsen et al. (2000). Sheep erythrocytes were washed twice with 9 mg/mL NaCl and once with veronal buffer (VB) pH 7.2 containing 2 mg/mL bovine serum albumin (BSA) and 0.02 % sodium azid (VB/BSA) and sensitized with rabbit anti-sheep erythrocyte antibodies (Viron amboceptor 9020, Ruschlikon, Switzerland). After incubation at 37°C for 30 min, the cells were washed as described above, and a 1% cell suspension in veronal buffer was prepared. The serum was diluted with VB/BSA to a concentration giving about 50% haemolysis. Samples were dissolved in VB/BSA (500 µg/mL) and a 4-fold dilution made. Sample dilutions (50 µL) and serum dilution (50 µL) were added in duplicates into the wells of a round bottom microtiter plate and incubated on a shaker at 37°C. After 30 min, the sensitized sheep erythrocytes (50 µL) were added and the microtiter plate incubated again as earlier described. After centrifugation at 1000 × g for 5 min, 100 µL of the supernatants were transferred to a flat bottom microtiter plate and the absorbance was read at 405 nm using a microplate reader. Hundred percent of lysis were obtained with distilled water and sensitized sheep erythrocytes. VB/BSA, serum and sensitized sheep erythrocytes were the control of the medium, and the pectin fraction, BPII from the leaves of *B. petersianum* was used as positive control. The prevention or inhibition of lysis induced by the test sample was calculated by the following formula:

$$\left[\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right] \times 100\%$$

A_{control} is the absorbance of control and A_{test} is the absorbance of test sample.

From these data, a dose-response curve was constructed and the concentration of test sample giving 50% inhibition of haemolysis

(ICH_{50}) was calculated. A low ICH_{50} value means a high complement fixing activity. This biological test system can have some day to day variations, and thus, the ratio $ICH_{50}\text{-BP11} / ICH_{50}\text{-sample}$ was calculated. A high ratio means high complement fixing activity.

Analysis of nitric oxide (NO) production

Nitric oxide (NO) released by activated macrophages is broken down to nitrite (NO_2^-) in the medium, which can be measured in a colorimetric assay using the Griess reagents. The mouse macrophage cell line Raw 264.7 was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, antibiotics, L-glutamine, and $5 \times 10^{-5} M$ 2-mercaptoethanol, and split every second day. Macrophages at a density of 5×10^5 cells/mL were seeded into 96-well flat-bottomed plates, and stimulated for 22 h in duplicates with increasing concentrations (1, 10, 100 $\mu g/mL$) of samples (Vd1, Vd2, SI1, Am1, Am2 and Ma1 selected from their effect in the complement fixing test), LPS (from *P. aeruginosa* 10, Sigma-Aldrich) and the pectic polysaccharide Oc50A1.I.A, from *O. celtidifolia* (Grønhaug et al., 2010) as positive controls, or medium alone. Nitrite was then determined in cell-free supernatants. The supernatant (50 μL) was mixed with an equal volume of Griess reagent A (1% [w/v] sulfanilamide in 5% [v/v] phosphoric acid) and incubated at room temperature in the dark for 10 min. After addition of 50 μL 0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride in water (Griess reagent B) the absorbance was read at 540 nm. A dilution series of $NaNO_2$ was used as a standard reference curve. The experiment was repeated two times, and the results shown are expressed as the mean \pm SEM.

RESULTS AND DISCUSSION

Polysaccharide fractions

Eleven polysaccharide fractions Vd1, Vd2, Vd3, SI1, SI2, SI3, Am1, Am2, Am3, Ma1 and Ma2 (Vd, SI, Am and Ma indicate the fractions respectively for *V. doniana*, *S. latifolius*, *A. mexicana* and Malarial-5[®]) were extracted as presented in Table 1. The fraction Vd3 presented the highest yield while Ma1 showed the lowest amount. Based on literature, this is the first time to extract polysaccharides from these plant species. These fractions with undetermined different molecular weights were the objects for further studies. These fractions could contain both neutral and acidic polysaccharides as they were not separated. This was not done as it was important to have all the water soluble materials present in the fractions that were tested for bioactivities, as those were as close as possible to those extracts prepared by the traditional healers.

Carbohydrate composition of the fractions

The monosaccharide compositions of the eleven fractions were determined and the results are presented in Table 2. The monosaccharide composition is typical for pectins, additionally, glucose was identified often present in plant material derived from water-soluble, neutral polysaccharides

like starch. Glucose was present in high amounts in all fractions. The other monosaccharides, galacturonic acid (Gal A), rhamnose (Rha), arabinose (Ara) and galactose (Gal), are all recognized as typical constituents in pectic polysaccharides (Inngjerdingen et al., 2012). The monosaccharides were mainly Gal A (39.9%), Ara (14.7%) and Gal (13.5%) for Vd1, while Vd2 had Gal (15.1%) as the major. The polysaccharide fraction SI1 had Gal (14.8%) and Ara (14.4%) as the major pectic monomers, Am1 had Gal (39.8%) and Ara (22.3%), while Am2 was rich in GalA (12.0%) and Rha (10.3%). The major pectic monosaccharides of the polysaccharide fraction Ma1 were Gal A (23.8%), Ara (23.7%) and Gal (22.5%). The presence of these monosaccharides proved polysaccharide existence in the investigated antimalarial plants and also that they could be of the pectic type polysaccharides (Inngjerdingen et al., 2012). The high content of glucose after methanolysis could be related to starch (Inngjerdingen et al., 2012). In addition the presence of fair amount of xylose in some samples could explain that some of the glucose also could be due to xyloglucan. All fractions contained arabinose and galactose, indicating the presence of arabinogalactans, polymers which are commonly present in pectin as side chains on the main core. In addition, the presence of galacturonic acid and rhamnose could indicate that the polymers may contain a main core consisting of a rhamnogalacturonan (indicative of RG I) linked with longer chains of homogalacturonan as noted by Bräunlich et al. (2018).

Immunomodulatory activities

Complement fixation activity

The ICH_{50} values of the polysaccharide fractions and the ratio $ICH_{50}\text{ BP11}/ICH_{50}\text{ sample}$ are given in Table 3 and Figure 1 respectively. The polysaccharide fraction (Am1) was the fraction with the highest activity, more potent than all the other polysaccharide fractions and approximately 8.3 times stronger than the standard polysaccharide (BP11 a pure pectic AG11 type polysaccharide isolated from *B. petersianum*). It is interesting to also note that the polysaccharide fraction (Vd1) had a relative high activity, approximately 3 times stronger than the standard polysaccharide (BP11). The fraction Ma1, isolated from the product Malarial-5[®], showed an effect in the complement assay twice times more than the one of the standard BP11, and SI1 had similar activity to the standard. Earlier investigations have shown that polysaccharides from Malian medicinal plants activated the complement system (Diallo et al., 2003; Inngjerdingen et al., 2006 and 2012; Togola et al., 2008; Austarheim et al., 2012; Zou et al., 2014a,b). It has also been shown that the ethyl acetate extract of *Biophytum umbraculum* (syn. *B. petersianum*) showed in vitro antiplasmodial effect and also an effect in the complement

Table 1. Yields of polysaccharides from the extractions.

Plant material	Polysaccharide	Yield (%)
<i>Vitex doniana</i> (leaves)	Vd1	0.3
	Vd2	0.5
	Vd3	4.2
<i>Sarcocephalus latifolius</i> (root bark)	SI1	0.4
	SI2	3.9
	SI3	0.4
<i>Argemone mexicana</i> (aerial parts)	Am1	0.8
	Am2	0.3
	Am3	0.3
Malarial-5® (herbal tea)	Ma1	0.1
	Ma2	0.4

Vd : *Vitex doniana*, SI : *Sarcocephalus latifolius*, Am : *Argemone mexicana*, Ma : Malarial-5®.

Table 2. Monosaccharide compositions (mol%) of polysaccharide fractions obtained from aqueous extracts of three antimalarial plants and the improved traditional medicine Malarial-5®.

Monosaccharide composition	Fraction										
	<i>V. doniana</i> (Vd)			<i>S. latifolius</i> (S)			<i>A. mexicana</i> (Am)			Malarial-5® (Ma)	
	Vd1	Vd2	Vd3	SI1	SI2	SI3	Am1	Am2	Am3	Ma1	Ma2
Ara	14.7	6.6	1.1	14.4	3.1	0.8	22.3	7.2	2.5	23.7	Traces
Rha	5.7	6.5	4.0	4.5	2.2	4.1	12.9	10.3	9.1	7.5	28.9
Xyl	1.8	7.2	2.7	2.4	3.1	1.8	2.8	9.4	17.8	2.0	3.4
Man	4.8	7.4	Traces	1.5	Traces	0.2	5.6	6.5	5.4	4.4	Traces
Gal	13.5	15.1	10.0	14.8	8.3	1.4	39.8	8.5	3.0	22.5	7.3
Glc	19.6	50.4	69.1	52.9	69.6	79.3	10.1	44.3	62.2	14.1	60.4
GlcA	-	-	-	0.9	-	1.8	-	1.8	-	2.0	-
GalA	39.9	6.8	13.1	8.6	13.7	10.6	6.5	12.0	Traces	23.8	Traces

Vd: *Vitex doniana*, SI: *Sarcocephalus latifolius*, Am: *Argemone mexicana*, Ma: Malarial-5®.

Table 3. ICH₅₀ values of the test samples.

Sample	BPII	Vd1	Vd2	Vd3	SI1	SI2	SI3	Am1	Am2	Am3	Ma1	Ma2
ICH ₅₀ (µg/mL)	19.9	6.3	38.9	150.6	22.4	186.3	54.9	2.4	27.1	63.3	10.5	77.5

assay (Austarheim et al., 2016).

Glucans are often recognized by their immunostimulatory activity and complement receptor type 3 (CR3, also CD11b/CD18) is a prime candidate as β -D-glucan receptor on human monocytes, neutrophils and NK cells (also dectin-1) according to Vannucci et al. (2013). The immunostimulatory activity of various polysaccharides include glucans, pectic polysaccharides, mannans, arabinogalactans, fucoidans, galactans, hyaluronans, fructans, and xylans as reported by Ferreira et al. (2015). The complement system is a potent player in innate immunity and a major effector arm of humoral immunity. Complement activation is linked to cellular

responses by the recognition of cleaved complement protein fragments by receptors on leukocytes and vascular cells. The three primary roles of complement in host defense against infection are to (1) activate an inflammatory response; (2) opsonize microbial pathogens for immune adherence; and (3) damage membranes, including lysis of susceptible organisms (Atkinson et al., 2019). Complement fixating activity has previously shown to be a good indicator for effect in the immune system by plant polysaccharides (Inngjerdigen et al., 2012). This test does not distinguish between activation and inhibition of the complement system, so we do not know if the samples have inhibited or activated the complement

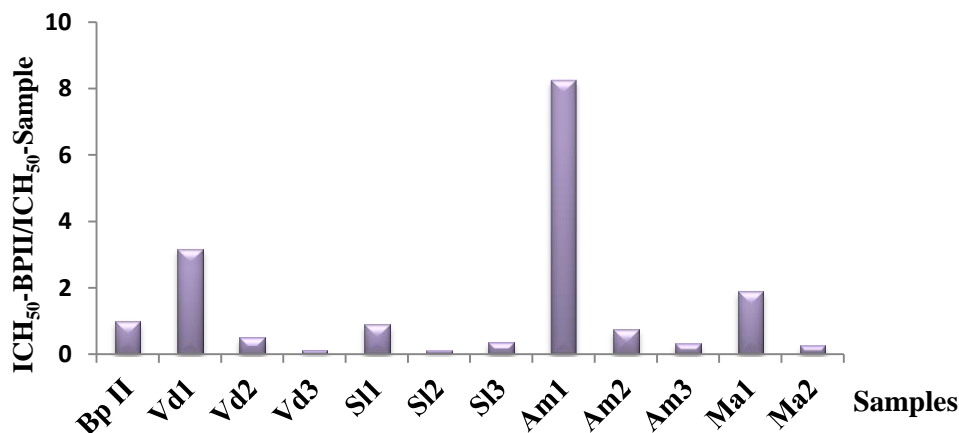


Figure 1. The complement fixation activity of the polysaccharide fractions, assayed as ICH₅₀ values of a polysaccharide standard BPII from *Biophytum petersianum* relative to ICH₅₀ of the polysaccharide tested (ICH₅₀-BPII / ICH₅₀-sample).

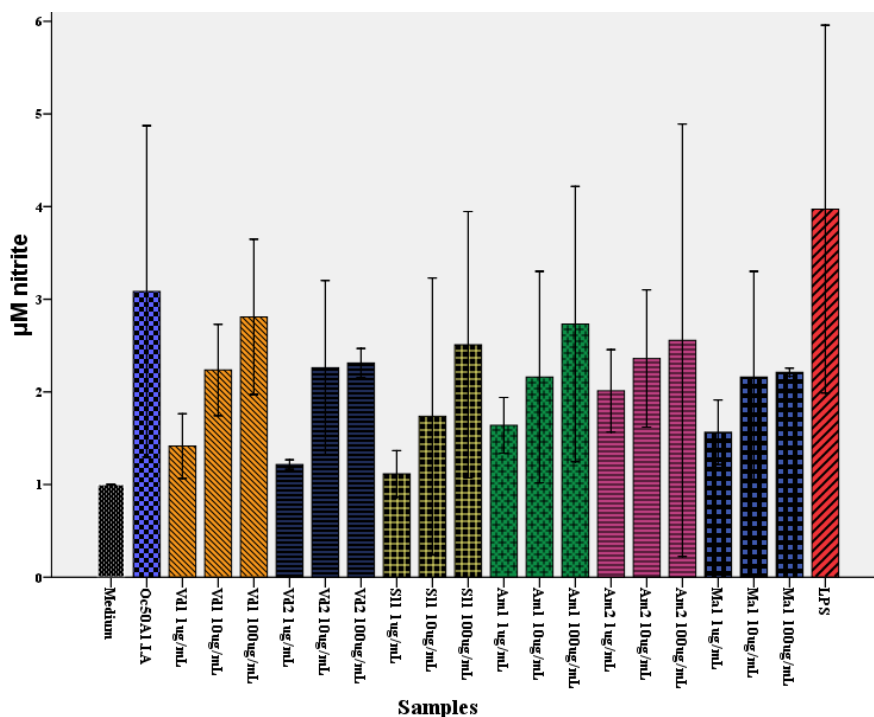


Figure 2. Stimulation of macrophages. A representative result is given as mean ± SEM. LPS and Oc50A1.IA from *O. celtidifolia* are present as positive controls.

system. Medicinal plants traditionally used against inflammatory diseases and wounds containing complement inhibitors have been reported (Cazander et al., 2012).

Measurement of nitrite oxide (NO) released from stimulated macrophages

The ability of the polysaccharide fractions to stimulate mouse macrophages to produce NO is shown in Figure

2. NO is a good marker for macrophage activation, and its stable breakdown product nitrite can easily be detected in culture supernatants. All fractions induced a dose dependent release of NO, as measured by the quantification of its breakdown product nitrite. Among the tested samples *A. mexicana* (Am1) and *V. doniana* (Vd1) showed the highest activities by inducing the release of 2.7 and 2.8 µM of nitrite from macrophages respectively at a dose of 100 µg/mL, while the positives controls Oc50A1.IA and LPS gave a release of 3.1 and 4.0 µM of

nitric oxide respectively. One of the important anti-parasitic chemicals generated by macrophages is nitric oxide (NO) during innate immune responses (Awasthi et al., 2003).

Plant-derived polysaccharides are potent immunomodulatory substances, and have been shown to be clinically therapeutics eg, lentinan. Previous authors reported that a variety of beneficial pharmacological effects of plant polysaccharides were attributed to their ability to modulate macrophage immune function (Yu et al., 2017). Some earlier studies supported the proposition that the production of nitric oxide by macrophages plays a crucial role in the control of parasitaemia at the initial periods of blood stage malarial infection (Awasthi et al., 2003). However, the ethyl acetate extract of *B. umbraculum* which revealed *in vitro* antiplasmodial effect, but gave an inhibition of macrophage activation (Austarheim et al., 2016).

Conclusion

All polysaccharide fractions from *A. mexicana*, *S. latifolius*, *V. doniana* and Malarial-5[®] contain pectic type polymers as well as glucans. These polysaccharides displayed immunomodulatory properties primarily as determined by the complement assay. The characterization of the polysaccharides acting on the immune system explains more the effectiveness of aqueous extracts and gives an additional justification for the traditional form. These results could be used as a new approach in the management of malaria. Therefore further investigations will be undertaken on *A. mexicana* that showed the highest immunomodulatory activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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