CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF WATER-SOLUBLE POLYSACCHARIDES FROM *Commiphora myrrha* (Nees) Engl. GUM

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Abstract. Myrrha, the gum resin exudate from *Commiphora myrrha* (Nees) Engl. tree, has been extensively used in traditional medicine for their beneficial effects. In this work, we isolated and purified water-soluble polysaccharide fraction (WSP), which afforded a yield of 42%. The chemical composition of WSP was mainly consisted of 83.33±2.63% carbohydrates, among them 79.25±1.06% are neutral monosaccharides and 13.4± 2.18% proteins, with traces of polyphenols (3.82%): The majority components of monosaccharides were galactose (45%) and arabinose (44%), with low levels of xylose (6%), and mannose (5%): In order to evaluate the immunostimulatory effect of WSP, phagocytic activity of opsonized human leucocytes against opportunist pathogen yeast, *Candida albicans*, was assessed. WSP could significantly ($p < 0.05$) enhance phagocytic capacity at the dose of 150 µg/ml, which showed a phagocytic activity of 29%. Moreover, the antihyperglycemic activity of extract was determined by α-D-glucosidase inhibitory activity, in vitro. WSP displays a promising inhibitory activity with an IC_{50} value of 8.30±0.12 mg/mL. The results of this work provide new knowledge in the field of chemical composition and biological activities of polysaccharides of *Commiphora myrrha* gum and might play an important role in activating phagocytosis leucocytes cells, thus having contribution to antidiabetic activity.

Keywords: Myrrha; traditional; gum; polysaccharide; biological activities.

INTRODUCTION

Commiphora myrrha (Nees) Engl. is a small tree or a large shrub, belonging to order Sapindales, family of Burseraceae, and genus *Commiphora*, which found in abundance in northeast Africa and the Middle East [3, 31]. There are over 200 species of *Commiphora* known, but *Commiphora myrrha* (Nees) is recognized as the best for its widespread use and for the quality of its myrrh, an aromatic gum resin, which is the stem resinous exudate of different *Commiphora* species [10, 16, 29]. The myrrh is one of the most frequently used gum resin in the prescriptions of traditional medicines in the world as a remedy for auto-immune diseases and inflammatory problems, such as rheumatic pains, amenorrhea, fever, stomach complaints, gall bladder, nephrosis syndrome, tumors, chest ailments, snake and scorpion bites, mouth ulcer and skin infections in ancient Egypt, India, China, Rome, Greece, and Babylon [4, 15, 22, 25, 26].

Preliminary researches have indicated that myrrh contained about 3–8% essential oil, 25–40% resins and 30–60% water-soluble gum [16, 32]. The myrrh contained many bioactive metabolites including flavonoids, terpenoids, steroids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives [4, 5]. The furanosesquiterpenoids hold in essential oil are acknowledged worldwide because of their beneficial uses, which have been found to possess antibacterial, antifungal, anesthetic, and antihyperglycemic properties [4, 12, 29]. Su *et al.* [29] was reported that ethanolic extract of the myrrh inhibited the development of paw swelling induced by formalin and reduced acetic acid-induced writhing response in mice at the weak doses. It has been also shown that, the pharmacological activities of the petroleum ether fraction decreased the levels of inflammatory factor PGE2 in the edema paw tissue. Al-Awadi *et al.* [2] have been shown that the extract of myrrh gum resin effectively increased glucose tolerance in both normal and diabetic rats. Tipton *et al.* [31] suggest that myrrh oil has anti-inflammatory properties, which determined myrrh oil effect on IL-1β-stimulated PGE2 production and NF-κB activation in gingival fibroblasts and epithelial cells. Furthermore, the furano -eudesma-1, 3-diene and 2-O-acetyl-8, 12- poxygermacra- 1(10), 4, 7, 11-tetraene, isolated by sequential extraction with aqueous ethanol and petroleum ether from the resin of *Commiphora myrrha* reported antihyperglycemic effect [33]. Zhu *et al.* [38] have isolated from *Commiphora myrrha* Six furanosesquiterpenoids, among of these compounds the epoxy-furanogermacr-10(15)-en-6-one which exhibited weak cytotoxic activities against a breast tumor cell in a clonogenic assay.

Most of the articles about myrrh have been focused on its resin and oil properties, such as chemical structure, pharmaceutical and therapeutic applications. Although, it has been demonstrated that myrrh is rich in polysaccharides, which may contribute to their biological functions. Just a few reports have only studied the chemical structure of polysaccharides [16], which shown that gum myrrh contains D-galactose, L-arabinose, and 4-methyl D-glucuronic acid in proportions 4: 1: 3, and approximately 18% of protein.

In this article, we will present for the first time the biological potential of water-soluble polysaccharides fraction extracted from *Commiphora myrrha* exudates produced by the secretory tissue in the stem bark after chemical analyzes. The aim of this study is to determine the composition and to evaluate the

antihyperglycemic and immunostimulatory effects of this fraction, which, to the best of our knowledge, has never been done so far.

MATERIALS AND METHODS

Raw material and chemicals. The gum resin from the stem of *C. myrrha* was purchased from the Algerian (Spice) Market, Ghardaia, Algeria and was identified as gum resin derived from *Commiphora myrrha* (Nees) Engl. by Prof. EDDOUD (Department of biology, Kasdi Merbah University, Ouargla, Algeria). The sample is referenced in the laboratory herbarium (Lot No. GCM-1808). May- Grünwald-Giemsa reagent, and PBS buffer were purchased from Sigma, Germany. Zymosan-A (*Saccharomyces cerevisiae* origin) were purchased from Fluka (Bio Chemika). Standard monosaccharides (including arabinose, rhamnose, xylose, galactose, glucose, mannose and galacturonic acid), metahydroxydiphenyl, trifluoroacetic acid (TFA). α-glucosidase, acarbose, and *p*-nitrophenyl α-D-glucopyranoside (*p*NPG) were purchased from Sigma-Aldrich (Germany). The human peripheral blood was obtained from healthy donors ranging from 20-35 years old. Whole blood was collected in a vacutainer consisting of NH sodium Heparin as anti-coagulant, during routine physical examinations from Ghardaïa province. This study was approved by the institutional review board of the Kasdi Merbah University (Ouargla, Algeria) and IBN ROCHD Medical Analysis Laboratory (Ghardaïa, Algeria), and all participants provided informed consent prior to blood sampling. All other used chemicals were of an analytical grade.

Polysaccharides extraction. The raw gum-resin of *C. myrrha* (10 g) was first exhaustively extracted with 100 mL of 96% ethanol, refluxing during 3 hours with stirring at 95°C. After centrifugation at 10000g during 15 min at room temperature, the insoluble residue was dried at 50°C in an oven overnight. Therefore, the pretreatment yield was 42.29%. A quantity of 1 g of dried insoluble residue was dispersed in 100 mL of ultrapure water at 80°C during 4 h under stirring (250 rpm). The mixture was centrifuged at 10000xg during 20 min at room temperature. The mixture was successively filtered through diatomaceous filters, and then was passed through a 5.0 µm GVS filter and Chromafil filters 0.45 µm. Three vol. of acetone were added and stored at -20°C during 24 h. The formed precipitate was centrifuged at 10000xg during 15 min at room temperature and washed 3 times with acetone and freeze-dried. This fraction called water-soluble polysaccharides (WSP).

Chemical composition of WSP. Moisture and ash content of WSP were measured by thermal gravimetric analysis realized between 30 and 900°C at a heating rate of 10°C/min under nitrogen. Total carbohydrate content was determined by phenol-sulfuric acid colorimetric method using glucose as a standard at 490 nm [11]. Total neutral sugar content was determined by the sulfuric resorcinol (1,3-dihydroxybenzen) method using glucose as a standard [23]. The quantitative estimation of uronic acid was carried out using m-hydroxydiphenyl assay with glucuronic acid as a standard [6]. The proteins content was estimated by the Coomassie Brilliant Blue G-250 method using bovine serum albumin as a standard [7]. Total phenolic compounds were determined by the Folin-Ciocalteu procedure using gallic acid as standard [28].

Determination of the monosaccharide's composition by GC/MS-EI. Ten mg of WSP were dissolved in 2 M HCl (2 mL) and heated at 90 °C during 4 h. The preparation was then evaporated under nitrogen stream. BSTFA: TMCS (99:1) derivatization was performed by adapting methods from Pierre *et al.* [24]. Trimethylsilyl-O-glycosides residues were solubilized by adding 500 µL of dichloromethane. Analyses were carried out by GC/MS-EI using an Agilent 6890 Series GC System coupled to an Agilent 5973 Network Mass Selective Detector. The solutions were injected into an Agilent HP-1 (30 m, 0.32 mm, 0.25 µm) at a helium flow of 2.3 mL/min. The helium pressure was 8.8 psi and the split ratio was 25:1. The rise in temperature was programmed for a first step at 100 °C during 3 min, an increment of 8 °C/min up to 200 °C for 1 min and then a final increment of 5 °C/min up to 250 °C. The ionization was performed by Electronic Impact (EI, 70 eV), the trap temperature was set at 150 °C and the target ion was fixed at 40-800 m/z.

Phagocytosis Assay. The phagocytic ability of polymorphonuclear leukocytes (PMNs) from human peripheral blood was measured using *Candida albicans* yeast. Briefly, *Candida albicans* (ATCC 2091) was inoculated into Sabouraud liquid broth from a stock culture maintained on a Sabouraud agar slope and left overnight at 30°C. The culture was centrifuged at 400g during 10 min and the supernatant was washed three times with phosphate-buffered saline (PBS) and incubated at 90°C during 1 h to kill *Candida*. The concentration of cells in PBS is estimated by measuring absorbance at 540 nm. The absorbance is adjusted to 1.0 which corresponds to 1 x 10^7 blastoconidia of *C. albicans* / mL, approximately [14, 30]. A total of 40 µL of WSP were mixed with 200 µL heparinized blood and incubated in closed shaking water bath at 37 °C for 60 min (60r/min). After that, tubes were put on ice to stop the reaction. Mixed samples were added with 40 µL of *C. albicans* at 0 °C. Samples were incubated in shaking water bath at 37 °C for 10 min, while for negative control; the samples were put on ice. After incubation, the samples were put on ice to stop reaction. Next, the samples were washed twice with 3 mL of PBS and were added 2 mL of lysing solution to lyse erythrocytes and incubated at room temperature during 20 min. Zymosan was used as a positive control. After lysing, PMNs were washed with PBS by centrifugation at 1000 rpm during 5 min three times and then fixed in ethanol. The fixed specimens were mounted on the glass slide and stained

with May-Grünwald-Giemsa. The cells with and without phagocytized yeasts out of a total of at least 100 cells were counted by direct visual enumeration using a light microscope (1000x).

In vitro α-D-glucosidase inhibitory activity assay. The inhibitory effect of WSP on α-D-Glucosidase activity was determined according to a previously reported method with slight modifications [35]. Commercial α-D-glucosidase inhibitor acarbose was used as a positive control. In brief, 10uL of polysaccharides extracts in the range of 2.5–10 mg/mL or standard (acarbose, 10uL) in range of 1–100 mM concentration was mixed with 125 uL of 4 mM *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG) solution (dissolved in 0.1 M pH 6.9 phosphate buffer) and 500 uL of 1U/mL enzyme solution was added. The reaction mixture was incubated for 10 min at 37°C and the reaction was stopped by adding 0.4 mL of 0.2 M Na_2CO_3. The enzyme activity was calculated measuring the absorbance of *p*NP released at 405 nm using the following formula for Zhang and Li [36]; (%) Inhibition= $(\Delta A_{\text{Negative control}} - A_{\text{Sample}}) / \Delta A_{\text{Negative control}}$.

Statistical analysis. The data shown are expressed as mean ± SD. Significance of difference was calculated by Student's t-test.

RESULTS

Chemical composition. The *Commiphora myrrha* gum is traditionally used in Algeria as a remedy for many inflammatory problems, but there is limited information on their chemical composition. Almost 42% of gum-resin was insoluble in ethanol. Chemical analysis revealed that this fraction (WSP) contained 83.33±2.63% of neutral monosaccharides, 9.4± 2.18% of proteins and low contaminated by phenolic compounds (< 1%), with 4.6 ± 0.1% moisture content and 3.73 ± 0.18% of ashes. it appeared to cause the high adhesivity observed in gum aqueous solutions.

Monosaccharide composition. The sugar analysis of WSP using GC/MS-EI revealed the following composition: galactose (45%), arabinose (44%), xylose (6%) and mannose (5%). The presence of uronic acid was not confirmed (Fig. 1). According to these results, the composition suggested that the water-soluble polysaccharides extracted from *C. myrrha* is an arabinogalactan -like polymer as described into literature for *Commiphora* gum. The ratio arabinose /galactose is close to 1.

Biological activities

Effect of WSP on phagocytic activity of polymorphonuclear leukocytes. One of the important notable features of polymorphonuclear leukocytes activation would be an increase in phagocytic activity [8]. To further investigate whether WSP stimulate immune system, we measured phagocytic activity of PMNs from human peripheral blood. Phagocytic activity of PMNs cells was examined by the uptake of opsonized *C. albicans*. The phagocytic index recorded after 10 min of incubation for *C. albicans* was different, according to the concentrations ranging from 50 µg/mL to 150 µg/mL with the respective result of negative control, zymosan, and WPS. In the negative control (absence of any treatment), the phagocytic index was 27±2 %, whereas a significant enhancement of phagocytic index was observed in PMNs treated with WSP (Table 1). The results show that a dose-dependent enhancement of phagocytic index was

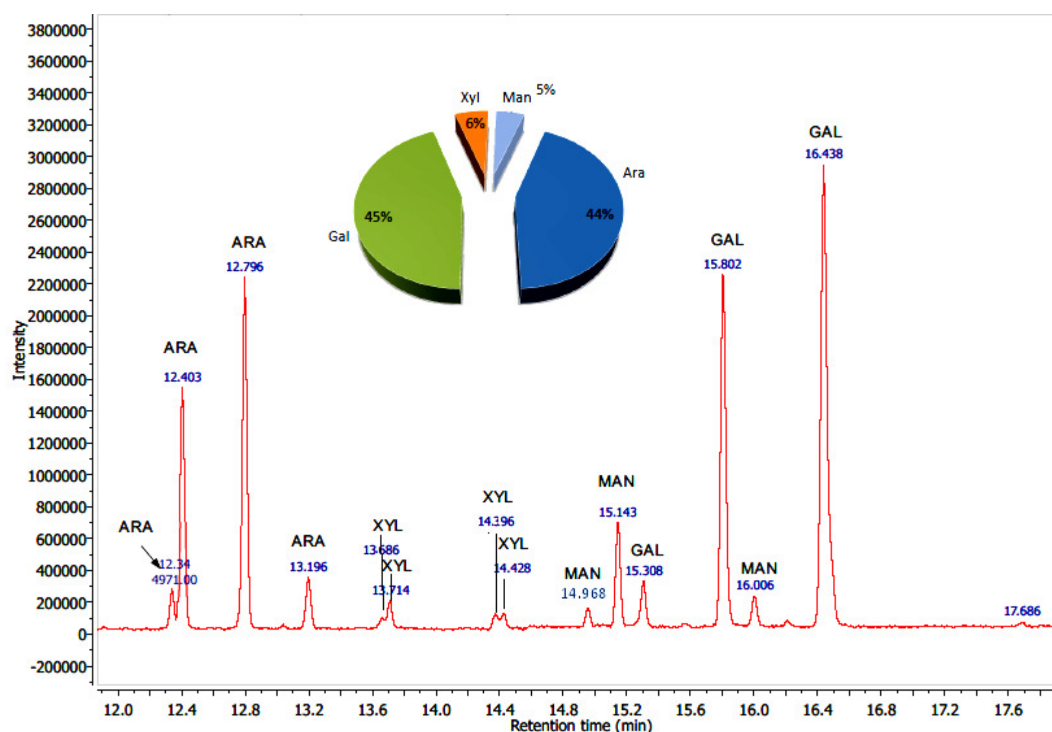

Figure 1. GC/MS-EI spectra of TMS-monosaccharides from the WSP

described in PMNs treated with 50–150 µg/mL doses of WSP, which were found a higher value (56±5 %) at the concentration of 150 µg/mL. The uptake of yeasts was activated by 3 %, 23 %, and 29 % in the concentration of 50 µg/mL, 100 µg/mL and 150 µg/mL of WSP, respectively, compared with the phagocytic index observed in negative control (26.6%). However, zymosan, the positive control, showed the highest stimulated activities, with phagocytic index of 70±3 at the 150 µg/mL concentration.

Table 1. Effects of WSP, zymosan, and negative control on the phagocytosis activity of PMNs by the uptake of opsonized *C. albicans*. The data are represented as mean ± SD; n = 5 (****P* <0.001; ** *P* < 0.01; * *P* <0.05).

Samples	Phagocytic index (%)		
	50 µg/mL	100 µg/mL	150 µg/mL
Zymosan	53 ± 5**	63 ± 3***	70 ± 3***
WPS	30 ± 4**	50 ± 4**	56 ± 5*
Negative Control	27 ± 2***	-	-

α-D-Glucosidase inhibitory activity. In Fig. 2, WSP had effect on inhibiting α-D-glucosidase activities but lower than acarbose's effect, a competitive type of α-D-glucosidase inhibition. IC_{50} was 8.30±0.12 mg/mL. As concentrations of WSP increasing from 2.5 to 100 mg/mL, inhibition rate of WSP increased, presenting a dose-effect relationship. When concentration of WSP was 25 mg/mL, inhibition rate was reached up to 70% and showed the maximum inhibition rate. Thus, the inhibitory effect didn't change much when the concentration reached 20 mg/mL.

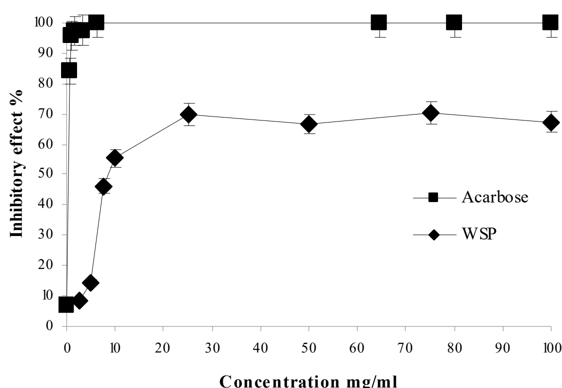

Figure 2. α-D-Glucosidase inhibition of WSP and acarbose for the catalysis of pNPG1

DISCUSSION

The present study confirmed results previously obtained on the proportions of gum and resin in the exudates from other *Commiphora* species which shown 30 to 60% of these exudates are water-soluble gum, and 25 to 40% are ethanol soluble resin. Hough *et al.* [18] show that the gum-resin of *C. myrrha* contains 40% of water-soluble polysaccharides among them 18% of protein. However, Dahi *et al.* [9] obtained 38±2.0 of water-soluble polysaccharides from *Commiphora Africana* gum, which contain 6.6 ± 0.1 of moisture (w%), 3.73 ± 0.01% ashes, 10.2 ± 0.6% proteins, 79.5 ± 0.6% carbohydrates.

Gums from different *Commiphora* species contain two major monosaccharides which are arabinose and galactose. Otherwise, the arabinose/galactose ratio, the type and the percentage of other monosaccharides are different. Hough *et al.* [18] reported that *C. myrrha* gum is mainly composed of galactose, arabinose and 4-methyl glucuronic acid with a ratio of 4: 1: 3. Also, Dahi *et al.* [9] find 47% arabinose and 28% galactose, 6.87% galacturonic acid, 4.96% Mannose, 4.61% xylose, 4.33% glucosamine and 4.24% fucose, with Ara/Gal ratio of 1.67. *Commiphora myrrha* gum has been shown to be a neutral arabinogalactan with traces of protein indicating that it is the arabinogalactan protein (AGP). According to published studies, AGPs consist of a large polysaccharide fraction and have a small peptide fragment, which suggests that arabinogalactan is a functional part of exudates [13, 21].

Many arabinogalactans isolated from medicinal herbs were reported to induce phagocytic activity of macrophages and polymorphonuclear leukocytes. An acidic arabinogalactan (AGP-2) from *Vigna radiata* could enhance the phagocytic capability of macrophages at concentration range of 1–50 µg/mL. Arabinogalactan protein from *Trachyspermum ammi* L. (Ajowan) displays significant immunomodulatory activity, at 1 µg/mL activated macrophages in releasing NO and significantly promoted phagocytosis [27]. The carbohydrate fraction contains 45.7% galactose, 34.5% arabinose, 7% glucose, 5% mannose and 4% xylose. Whereas deproteinized AGP or deglycosylated AGP showed compromised efficiency. Therefore, the structural complexity protein-glycan plays a crucial role in activating the immune response that mainly functions on the molecular pattern recognition mechanism [27]. Igor *et al.* [19] isolated five arabinogalactan type II fractions from the cones of *Juniperus scopolorum*, which shown that the high molecular weight polysaccharide fractions had potent immunomodulatory activity. These polysaccharide fractions stimulated macrophages for an enhanced NO production via induction of nitric oxide synthase, and induced macrophages to secrete both inflammatory (IL-1, IL-6, TNFα, and IL-12) and anti-inflammatory (IL-10) cytokines. An immunomodulatory arabinogalactan named ICPA was isolated from *Ixeris chinensis,* mainly composed of galactose and arabinose with minor amount of glucose. Their molecular weight was 58.1 kDa. ICPA enhanced the phagocytic activity and the secretion of NO, TNF-, and IL-6 by RAW 264.7 cell in the concentration range of 100 to 400 µg/mL, in a dose-dependent manner, which shown very low phagocytic activity compared to that of WSP [17]. Although *Commiphora* AGP shows similarities to AGPs with respect to immunomodulatory activity, it stands different when compared to the phagocytosis capacity.

A large group of arabinogalactans have strong immunomodulatory properties and show biological activity against various chronic diseases, such as diabetes mellitus. AGP mechanism involved in antidiabetic activity may include inhibition of intestinal α-D-glucosidase enzymes which improved tolerance of glucose. Bisht *et al.* [5] showed that IC_{50} of α-D-glucosidase inhibitory was 0.5 mg/mL of polysaccharides isolated from *Acacia tortilis* plant gum exudate. It consisted of 78.1% L-Arabinose, 18.64% D-galactose, 0.60% D-glucose, 1.71% L-rhamnose, 0.74% D-mannose, 3.88% D-galacturonic acid, and 4.35% D-glucuronic acid [1]. However, Wang *et al.* [34] found the stronger inhibition of α-D-glucosidase at 0.8 mg/mL of the heteroglucan, extracted from *Fagopyrum tartaricum*. Moreover, Zhang *et al.* [37] revealed that polysaccharides rich in arabinose, exhibited remarkable α-D-glucosidase inhibition activities. Among three polysaccharide fractions isolated from *Rhynchosia minima* root, Jia *et al.* [20] shown that the richest fraction of arabinogalactan has the strongest inhibition of α-D-glucosidase activity, with IC_{50} values of 8.85 mg/mL, closer to that of WSP.

The principal findings are that the exudate gum resin of *Commiphora myrrha* containing about 42% of total polysaccharide content. It is one of the rich source of arabinogalactan which composed of 45% Gal, 44% Ara, 6% Xyl, and 5% Man. *C. myrrha* gum resin contains water soluble polysaccharides, which may endow the myrrh with strong inhibitory activity against α-D-glucosidase. Therefore, suggested that WSP could be a promising therapeutic agent for the management of blood glucose, which be further developed in the health food industry. Moreover, WSP had potent immunomomodulatory properties in enhanced phagocytosis of PMNs. The WSP concentrations have significant linear correlations with α-D-glucosidase inhibition and phagocytic ability of polymorphonuclear leukocytes. Polysaccharides have been confirmed to be one of the major active ingredients responsible for health-promoting of the myrrh. It is well-known that polysaccharides effect may depend on glycosidic linkage, molecular weight, conformation, and degree of branching. Thus, is strongly recommends further investigation of myrrh polysaccharides structure information.

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