Full Length Article

Non-Specific Inhibitor from *Origanum vulgare* Leaves Restrains *Porphyromonas gingivalis* Growth and Virulence Factors

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Abstract

The bacterium *Porphyromonas gingivalis*, a major pathogen causing chronic periodontitis, secretes several peptidases and hemagglutinins as major virulence factors. Inhibition of these virulence factors is important to prevent periodontal diseases. The crude aqueous extracts from 38 plant organs of 25 plant species were screened to inhibit hemolysis, hemagglutination, gingipains and bacterial growth. Although, several plants inactivated virulence factors (hemolysis, hemagglutination, and gingipains) independent of cell growth suppression, only three plants namely *Origanum vulgare* (leaves), *Glycyrrhiza glabra* (areal parts) and *Salvia offinalis* (areal parts) hindered cell growth and virulence agents. Among them, the aqueous extract of *O. vulgare* provided low MIC values for heme aggregation, erythrocyte lysis and gingipain. The inhibitory compound from *O. vulgare* leaves was isolated and characterized as a hydrolysable tannin-like compound that can inhibit *P. gingivalis* growth and virulence factors non-specifically. The results of the current study will enhance the application of *O. vulgare* to prevent periodontitis and support oral hygiene. © 2020 Friends Science Publishers

Keywords: Hydrolysable tannin; *Origanum vulgare*; Periodontist; *Porphyromonas gingivalis*

Introduction

Oral diseases are important and common chronic ailments (Kadowaki *et al*. 2004; Peres *et al*. 2019). Of them, periodontal disease (periodontitis) is caused by bacterial inflammation of the tissues supporting the teeth and is a major oral health problem. Periodontal disease causes different symptoms and can result in the loss of teeth. Additionally, recent studies have demonstrated a strong association between periodontal disease and serious systemic diseases such as diabetes, atherosclerosis, stroke and coronary heart diseases (DeStefano *et al*. 1993; Beck *et al*. 1999, 2017).

Many different bacteria exist simultaneously in the oral cavities of patients with periodontal disease. In particular, *Porphyromonas gingivalis* is a Gram-negative, asaccharolytic, obligate anaerobic rod bacterium and appears to be the prime etiological agent in the pathogenesis and progression of the inflammatory events underlying periodontal disease (Gron *et al*. 1997; Kan *et al*. 2019). *P. gingivalis* requires exogenous amino acids, porphyrin, and iron for growth and virulence and also produces a number of virulence factors such as hemagglutinins and gingipains. Hemagglutinins attach to the host tissues and lyse erythrocytes to uptake Fe ions as an essential nutrient; peptidases degrade connective-tissue proteins into small peptides and amino acids for use in growth and metabolism. In addition, gingipains are cysteine proteinases in *P. gingivalis* that are believed to be major virulence factors (Snipas *et al*. 2001; Lamont *et al*. 2018). Compounds that suppress the adhesion of *P. gingivalis* to teeth by suppression of these virulence factors represent a new strategy for preventing periodontitis (Cutler *et al*. 1995; Kan *et al*. 2019).

Pathogenic bacteria are increasingly resistant to currently used antibiotics and chemotherapeutic medications and thus medicinal plants are being investigated for alternative treatment options for oral diseases. The use of medicinal plants is generally safe, and they are abundant, inexpensive, and cost-effective, particularly in developing countries. Medicinal plants are a treasure trove of biologically active compounds, many of which have acted as lead compounds for the development of pharmaceuticals (Cheesman *et al*. 2017).

In Sudan, people have been using plant remedies to treat illnesses since ancient times. Many studies have verified the utility of Sudanese medicinal plants for treating various diseases, including bacterial and fungal infections. However, little has been reported to date regarding the inhibitory activities of Sudanese plants on the growth of *P. gingivalis* (Mohieldin *et al*. 2017). To the best of our knowledge, the inhibition activities of Sudanese medicinal plants against hemagglutination, hemolysis, and gingipain has not been investigated. Furthermore, several researchers have hypothesized that inhibition of gingipains is independent of cell growth suppression (Kushiyama *et al*. 2009; Niehues *et al*. 2010; Feghali *et al*. 2012; Messing *et al*. 2014). Therefore, in this study, we focused on blocking the primary phases of bacterial adhesion to the host by preventing hemolysis, specifically through the inhibition of hemagglutinins and proteases that provide nutrients to *P. gingivalis* for their growth and survival. The inhibitory activities of the aqueous extracts of 38 plants were evaluated against hemagglutinins, hemolysis, gingipain and bacterial growth. Among them, *Origanum vulgare* suppressed all virulence factors. Following phytochemical analysis, the inhibitory activity of isolated and related bioactive compounds was investigated.

Materials and Methods

Preparation of plant aqueous extracts

Plant samples were collected from different areas in the state of Khartoum in Sudan. Cleaned and powdered plant materials were extracted three times with distilled water (ratio of 1 g sample to 10 mL water) for 30 min at room temperature. The supernatants were collected by centrifugation, and then filtered and lyophilized. The yields of crude extracts are listed in Table 1. The plant extracts were stored at −20°C and used for bioassays.

Cultivation of *P. gingivalis*

P. gingivalis strain TDC60 (Watanabe *et al*. 2011) was provided by the RIKEN BRC through the National Bio-Resource Project of MEXT, Japan. The strain was grown in tryptic soy blood agar medium Agar Base EH; Difco[TM], (Becton, Dickiton and Company, France), supplemented with hemin (5 mg mL^{-1}), menadione (1 mg mL^{-1}) and horse blood at 37°C under anaerobic conditions for 4–5 days (Gao *et al*. 2010; Rangarajan *et al*. 2017). The cells were then inoculated into 5 mL GAM broth medium and incubated anaerobically at 37°C for 2 to 3 days until the bacteria reached late stationary phase of bacteria growth as evaluated spectrophotometrically at 600 nm. The cells were harvested by centrifugation and the culture supernatant (Pg-sup) was used for inhibition assays.

Hemagglutination and hemolytic inhibition assays

Erythrocyte aggregation (hemagglutination) and hemolytic assays were performed as described previously (Saiki and Konishi 2007) with minor modifications. Horse defibrillated blood (Cosmo Bio Co., Ltd., Tokyo, Japan) was rinsed three times with phosphate-buffered saline (PBS) and centrifuged for 5 min at $2000 \times g$. The washed erythrocytes were diluted to 5% (v/v) with PBS prior to use.

For the hemagglutination test, 80 μL PBS, 10 μL Pg-sup, and 10 μL plant extract were mixed in a 96-well, round-bottom microtiter plate and shaken well. After incubation for 10 min at room temperature, 100 μL of the 5% washed horse erythrocyte suspension was added. Hemagglutination was evaluated visually after incubation at room temperature for 2 h. For the hemolysis assay, 80 μL PBS, 10 μL Pg-sup, and 10 μL plant extract were mixed in a 96-well flat-bottom microtiter plate and shaken well. After incubation for 10 min at room temperature, 100 μL of the 5% washed horse erythrocyte suspension was added. The plate was incubated at 37°C for 2 h and centrifuged at $2000 \times g$ for 5 min, after which 100 μL supernatant was transferred to a clean plate and liberated hemoglobin was observed visually.

For both assays, PBS was used instead of Pg-sup and plant extract as the negative control. The minimum inhibitory concentration was calculated from the final concentration of the lowest dilution exhibiting complete inhibitory activity.

Protease inhibition

The protease inhibitory activity of plant extracts was determined as described previously (Kariu *et al*. 2017). Compounds exhibiting activity inhibitory to gingipains were identified using *t*-butyloxycarbonyl-L-valyl-L-prolyl-L-arginine-4-methylcoumaryl-7-amide (Boc-VPR-MCA) as a protease substrate. Ten microliters of Pg-sup and 10 μL of plant extracts were added into the mixture containing 40 μL of 0.5 *M* Tris-HCl buffer (pH 7.5) and 30 μL of distilled water contain 5 m*M* of L-cystein. After 5 min preincubation at 37°C, 10 μL of 500 μ*M*Boc-VPR-MCA was added to the mixture. The release of aminomethyl-coumarin was measured with an excitation at 380 nm and emission at 440 nm using a fluorescence spectrophotometer (Infinite M 200 Pro, TECAN, Männedorf, Switzerland, Japan). The linear increase of aminomethyl-coumarin release was recorded for 5 min in the presence or absence of aqueous crude extracts at different concentrations and the half maximal inhibitory concentration (IC_{50}) was calculated from the obtained dose-response curve.

P. gingivalis growth inhibition

The influence of the plants' aqueous extracts on *P. gingivalis* growth was investigated by measuring the

turbidity of bacterial suspension in a 96-well microplate. Ten microliters of plant extracts (20 mg mL^{-1}) was added to 200 μL of *P. gingivalis* suspension standardized at 2 x10^7 CFU (Kariu *et al.* 2017). The plates were incubated anaerobically at 37°C for 60 h. The turbidity was then measured at 600 nm via a microtiter plate reader (Model 680, Bio-Rad Laboratories Inc., Hercules, C.A., U.S.A.).

Isolation of biologically active compounds

The extraction of the inhibitory active constituents from *O. vulagare* leaves was performed according to the method described by Mendonça-Filho (2006). About 40 g of dried and powdered leaves was extracted three times with 400 mL distilled water for 30 min at room temperature. The soluble extracted compounds were collected by filtration followed by centrifugation. The extract was portioned between distilled water and ethyl acetate. The water phase was fractionated using methanol precipitation (30% methanol for 20 min). The supernatant of methanol which had the highest activity for inhibition of hemagglutination, hemolysis and the protease, was fractionated using an ODS column (Cosmosil 75 C18-OPN; Nacalai Tesque, Kyoto, Japan) eluted first with MeOH in H$_2$O (0–80%, 20% increments) and then absolute methanol. The 40% methanol fraction exhibited the highest activity and was subjected to silica gel column chromatography (Daisogel IR-60-63/210; Daiso, Osaka, Japan) by eluting first with acetone/hexane (0–100%), then 100% methanol, and finally distilled water. The compounds in the water eluate were fractionated using a C18 Sep Pak column cartridge (Sep-Pak C18 20 cc Vac cartridge, 5 g sorbent per cartridge, 55–105 μm particle size; Waters, USA). The column was conditioned with MeOH and equilibrated with distilled water, then eluted with H$_2$O/MeOH (0, 20, 40, 60, 80 and 100% methanol) to give six subfractions. The 60% methanol fraction exhibited the greatest inhibitory activity and was injected into a high-performance liquid chromatograph (HPLC) equipped with an ODS column (Cosmosil 5 C18-AR-II, 4.6 ID □ 150 mm; Nacalai Tesque) and eluted using the following analytical conditions: gradient elution program, 5–80% B/(A + B) within 60 min; solvents, milli-Q water (A) and acetonitrile (B); flow rate, 0.8 mL/min; column temperature, 40°C; detection, UV 280 nm.

Characterization of polymer-like compounds

The Prussian blue test was used to detect phenolic compounds as previously described (Price and Butler 1977). Thin layer chromatography (TLC) on silica gel 60 F254 aluminum sheets (Merck, Darmstadt, Germany) was conducted to identify the types of large polyphenolic compounds isolated using 2-methyl-2propanol *tert*-butanol–acetic acid–water (3:1:1, v/v) as the mobile phase (Harborne 1973). The compounds were detected using UV at 254 nm. Matrix-assisted laser desorption ionization–time-of-flight

mass spectrometry (MALDI-TOF MS) was used to analyze the tannin (I. Tannin) isolated from *O. vulagare* and standard tannic acid obtained from Sigma-Aldrich (St. Louis, MO) using 2,5-dihydroxybenzoic acid as the MALDI matrix. I. Tannin (10 μL) (1.0 mg mL^{-1}) and tannic acid (1.0 mg mL^{-1}) were separately mixed with the matrix solution, and 1.0 μL was dropped onto the target plate and dried. The samples were analyzed using an Auto-flex TOF instrument (Bruker Daltonics Inc., Billerica, MA, USA).

Structural study

Cysteamine degradation was assessed as previously reported (Zhang and Lin 2008). I. Tannin (50 μL, 4.0 mg mL^{-1}) was dissolved in 50 μL of 3.3% hydrochloric acid in methanol, added to 100 μL of cysteamine hydrochloride in methanol (50 mg mL^{-1}), and heated at 40°C for 30 min. After cooling to room temperature, the solution was filtered (Φ13, 0.22 μm, Merck) and 10 μL of the sample solution was analyzed by HPLC. The remaining I. Tannin sample was partially acid hydrolyzed using a method described previously (Tanaka *et al.* 1986) by heating at 100°C in 5% sulfuric acid for 10 h. The solution was filtered and a 10 μL aliquot was analyzed by HPLC using the following analysis conditions: elution solvents, 0.1% formic acid (A) and acetonitrile contain 0.1% formic acid (B); gradient elution (3–60%) over 10 min; column, Cosmosil C18, (ø 1.7 μm × 50 mm); temperature, 40°C.

Results

Inhibitory activities of plant extracts against virulence factors in *P. gingivalis*

In this study we investigated 38 organ parts from 25 plant species belonging to 17 families. Our selection was based on the historical and traditional uses of plants and plant parts in Sudanese folk medicine. The potential inhibitory activities of aqueous extracts of these samples against *P. gingivalis* activities associated with periodontal disease were evaluated in a 96 well plate. The minimum inhibitory concentration (MIC) of each sample as determined by hemagglutination and hemolysis assays, and the IC$_{50}$ value for protease inhibition, are summarized in Table 1. Except for the aqueous extract of *Grewia tenax* and *Ziziphus spina-christi* fruits, all tested samples exhibited inhibitory activities toward at least one tested parameter. Among the plant extracts, 23 showed inhibitory activities against hemagglutination with MIC values of 0.06–4.0 mg mL^{-1}, 32 inhibited hemolysis with MIC values of 0.03–4.0 mg mL^{-1} and 16 plant extracts inhibited protease activity with IC$_{50}$ values of 0.11–1.70 mg mL^{-1}.

All plant extracts were tested at 1.0 mg mL^{-1} for antibacterial activity against *P. gingivalis*. Ten of the 38 extracts exhibited inhibitory activity, with four extracts suppressing growth 81–100%, two suppressing growth 61–

Table 1: The inhibitory activities of the aqueous extracts from selected Sudanese medicinal plants against *Porphyromonas gingivalis* virulence factors

Scientific name	Used part	Yield (%)	MIC (mg mL^{-1})		IC$_{50}$ (mg mL^{-1}) Gingipain	Growth inhibition at 1.0 mg mL^{-1}
			Hemagglutination	Hemolysis		
Tamarindu sindica	F	72.9	ND^c	4	ND	-
	L	22.2	ND	1	0.14	-
	S	19.7	ND	0.03	1.03	-
Salvadora persica	B	22.4	0.5	0.5	0.28	-
	L	38.3	4	0.5	ND	-
	M	12.6	4	0.5	ND	-
Erythrina abyssinica	S	9.06	ND	ND	0.13	-
Origanum vulgare	L	21.1	0.25	0.25	0.34	+
Ambrosia maritima	A	15.1	ND	4	0.25	+
Acacia nilotica	D	15.5	ND	0.03	0.28	-
Guiera senegalensis	L	10.3	0.5	0.5	ND	-
Grewiatenax	F	62.9	ND	ND	ND	-
	S	30.1	0.5	4	ND	-
Solenostema argel	L	45.5	4	1	ND	++
Trigonella foenum-graecum	S	27.1	1	4	0.35	-
Balanites aegyptiaca	F	79.6	1	0.5	ND	+-
	L	34.0	ND	ND	ND	+-
	P	34.8	1	1	ND	+-
Glycyrrhiza glabra	A	16.1	1	4	1.70	++
Ceratonia siliqua	F	52.7	ND	4	ND	-
	S	21.2	4	4	ND	-
Acacia oerfota	M	11.1	4	4	ND	-
Khaya senegalensis	L	20.7	ND	1	ND	-
Cymbopogon schoenanthus	L	8.1	ND	1	ND	-
	A	14.8	4	1	ND	++
Monechma ciliatum	S	10.8	0.06	4	0.11	-
Salvia offinalis	A	19.5	4	0.25	0.18	++
	L	19.7	4	4	ND	-
Azadirachta indica	L	29.4	ND	4	ND	-
Lepidium sativum	S	19.2	0.5	4	ND	-
Cyperus rotundus	A	81.3	4	0.5	0.53	-
	Z	6.1	4	1	ND	-
Leptadenia arborea	L	36.5	1	1	0.72	-
Ziziphus spina-christi	F	59.5	ND	ND	ND	-
	L	19.4	0.06	ND	0.14	-
Acacia seyal	M	21.6	ND	0.03	0.96	-
	B	30.1	ND	0.5	0.35	-
Capparis deciduas	M	13.9	4	ND	ND	+-

A aerial part, B bark, D pod, L leaf, M stem, P peel, S seed, F fruit, Z rhizome
Yield %: based on dry weight
ND: not detectable inhibition activity
++ 81–100%, + 61–80%, ±50–60%, - < 50% inhibition rate

80%, and four extracts suppressing growth 50–60%. Although, several plant extracts showed potent inhibitory activities against virulence factors or cell growth independently, only, the leaf extracts of *O. vulgare* and aerial part extracts of *Glycyrrhiza glabra* and *Salvia offinalis* suppressed all the tested virulence factors and bacterial growth. Among these three plants, the aqueous extract of *O. vulgare* leaves exhibited the most potent inhibitory activity against all tested virulence parameters, and this sample was selected for further investigation and purification.

Identification of inhibitory compound in the aqueous extract of *O. vulgare* leaves

The compounds in the *O. vulgare* leaf extract exhibiting inhibitory activity on virulence factors was extracted and identified as described above. HPLC analysis of purified aqueous extract of *O. vulgare* leaves provided a single, broad polymer-like peak (Fig. 1a). Because of its heat resistance, high water solubility, and broadness of the single peak, we speculated that the active fraction harbors a polyphenolic or tannin-like polymeric compound. Colorimetric reactions of this active fraction and tannic acid with Prussian blue provided a dark blue to turquoise color, indicating that the isolated fraction contains a large polyphenolic or tannin-like compound (Fig. 1b). We verified this possibility by TLC analysis of the isolated fraction and standard tannin and found that the isolated fraction did not contain procyanidin oligomers and that the phenolic polymer provides a TLC profile similar to that of the tannic acid standard (data not shown).

The isolated compound and tannin standard were analyzed using MALDI-TOF MS. The molecular mass of

Table 2: Inhibitory activity of the isolated tannin-like polymer against *P. gingivalis* virulence agents

Compounds	MIC (mg mL^{-1})			IC$_{50}$ (mg mL^{-1})
	Hemagglutination	Hemolysis	Growth	Gingpain
Isolated tannin	0.0063	0.0063	0.05	0.05
Tannic acid	0.0063	0.0032	0.15	0.05
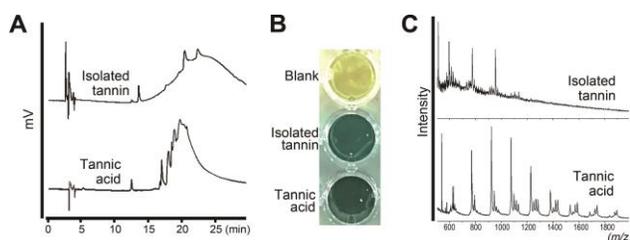
Fig. 1: Characterization of polyphenolic compound isolated from *O. vulagare*. **(A)** HPLC chromatogram of the tannin-like compound and tannic acid. **(B)** The reaction of the isolated polyphenolic compound and tannic acid with Prussian blue. **(C)** MALDI-TOF MS analysis of tannin-like compound and tannic acid
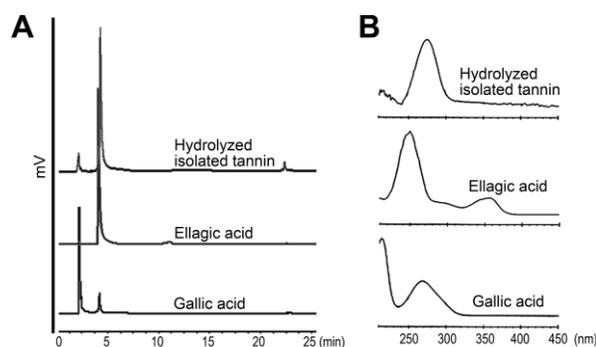
Fig. 2: HPLC chromatogram **(A)** of the acid-hydrolyzed tannin-like compound and UV spectrum of the single peak **(B)** compared to that of ellagic and gallic acid

the isolated compound ranged from 509 to over 1000 and that of tannic acid from 530 to over 1500. The mass spectrum of the isolated fraction was similar but not the same as that of tannic acid: both showed peaks at similar intervals of 179 mass units for the isolated compound and 152 mass units (corresponding to gallic acid) for tannic acid (Fig. 1c). The isolated tannin-like polymer was found to be stable against chemical degradation by cystamine (data not shown), confirming that the isolated compound is hydrolysable tannin.

Identification of the monomer of the isolated tannin-like compound (I. Tannin) was carried out by subjecting it to acid degradation, followed by HPLC and absorbance spectrum analysis. Comparison of the HPLC chromatograms of the degraded isolated tannin-like compound with those of ellagic and gallic acids showed that the single peak of I. Tannin at 5 min corresponded to that of ellagic acid (Fig. 2a). The UV spectrum of I. Tannin was somewhat similar to that of ellagic acid (Fig. 2b).

Inhibitory activity of the isolated compound

The isolated tannin-like compound at 0.05 mg mL^{-1} exhibited inhibitory activity against all tested periodontal factors, including *P. gingivalis* growth, hemagglutination (MIC: 0.0063 mg mL^{-1}), hemolysis (MIC: 0.0063 mg mL^{-1}), and protease (IC$_{50}$: 0.05 mg mL^{-1}) (Table 2). Tannic acid also exhibited inhibitory activity against hemagglutination, hemolysis, and protease comparable to that of I. Tannin but the antibacterial activity of I. Tannin was 3-fold higher than that of tannic acid.

Discussion

The aqueous extracts from 38 plant organs from 25 plant species used in folkloric Sudanese medicine were evaluated for inhibition of virulence factors (hemagglutination, hemolysis, and protease activity) and for suppression of growth of *P. gingivalis*. Gingipains adhere to host cells to obtain the nutrients required for bacterial survival and the production of virulence factors. In the primary stages of inflammation, *P. gingivalis* secretes hemagglutinin and proteases which form large multifunctional complexes known as hemagglutinin/adhesion domains. These domains engage in proteolysis and are involved in the adhesion of host cells to red blood cells for acquisition of heme through hemagglutination and hemolysis (Kadowaki *et al.* 2004; Tezuka *et al.* 2006; Guo *et al.* 2010; Kan *et al.* 2019). Several studies have, therefore, suggested targeting these virulent factors to block the primary stages of *P. gingivalis* pathogenicity, using natural, safe, and effective therapeutic agents (Nakayama *et al.* 1996; Kadowaki *et al.* 1998; Lu *et al.* 2019). Gingipains are obvious targets as their activity is independent of bacterial cellular programs and cell viability.

Most of the tested aqueous extracts inhibited one or more virulent proteins but only 10 plant extracts suppressed bacterial growth. This suggests that the inhibition of bacterial growth in a liquid medium and the outer cell membrane virulence of gingipains are achieved through different inhibition mechanisms. Similar findings were obtained using tea catechin, lactoferrin, cranberry proanthocyanidin, and prenyl flavonoids, all of which inactivated gingipains independent of bacterial growth suppression (Kushiyama *et al.* 2009; Niehues *et al.* 2010; Feghali *et al.* 2012; Messing *et al.* 2014). In contrast, leaf extracts of *O. vulgare* and aerial part extracts of *G. glabra* and *S. offinalis* suppressed gingipains and *P. gingivalis* growth, suggesting that they may harbor ideal inhibitors for all *P. gingivalis* virulence factors. No ideal periodontal inhibitor has been reported to date, thus further analysis to identify biologically active compounds in these plant extracts is essential.

Comparison of the MIC values of the three plant extracts (*O. vulgare*, *G. glabra*, and *S. offinalis*) showed that *O. vulgare* exhibited low MIC against all tested parameters especially for hemolysis and hemagglutination (Table 1).

Hemagglutination is vital for *P. gingivalis* to adhere to host cells in gingival tissues and to uptake heme and iron by the aggregation and lysing of erythrocytes, resulting in the accumulation of dental plaque and the secretion of other virulent factors (Guo *et al*. 2010). Several natural and non-natural products have been reported to reduce *P. gingivalis* pathogenicity by blocking bacterial adhesion and decreasing proteolytic activity (Curtis *et al*. 2002; Yokoyama *et al*. 2007; Löhr *et al*. 2011; Kan *et al*. 2019; Kariu *et al*. 2019). Since *O. vulgare* is used worldwide primarily as a food additive due to its pleasant aroma (Illias *et al*. 2018), we therefore, focused on aqueous extracts of *O. vulgare* leaves and succeeded in isolating a tannin-like compound as a non-specific inhibitor against all virulence agents.

Tannins are widespread in plants and in plant-based foods and are classified into two groups. The first group is hydrolysable tannins (HTs), which are esters of phenolic acids and a polyol, usually glucose. The phenolic acids are either gallic acid (in gallotannins) or phenolic acids derived from the oxidation of galloyl residues (in ellagitannins). The second group of tannins is proanthocyanidins (PAs), which are the most common type of tannin in our diet (Santos-Buelga and Scalbert 2000; Smeriglio *et al*. 2017). Treatment with cystamine does not affect isolated tannins, perhaps due to the C-C or C-H linkages in tannins and their degree of polymerization. However, in this study, sulfuric acid degradation followed by HPLC analysis of the isolated and standard tannins, provided a single peak with a retention time and UV spectrum parallel to that of ellagic acid with a slight difference. This result indicates that the isolated tannin might be a derivative of ellagic acid and may be a new type of very polar HTs.

The isolated tannin-like compound exhibited high inhibitory activity against hemagglutination (MIC 0.0063 mg mL^{-1}), hemolysis (MIC 0.0063 mg mL^{-1}), protease activity (IC$_{50}$ 0.05 mg mL^{-1}), and bacterial growth (MIC 0.05 mg mL^{-1}). These findings are in agreement with reports of antimicrobial properties of HTs in extracts from edible and non-edible plants (Santos-Buelga and Scalbert 2000; Buzzini *et al*. 2008; Pinelli *et al*. 2015; Smeriglio *et al*. 2017). Several studies have reported an association between a reduction in polyphenolic compounds from green tea and cranberry polyphenols and an increase in dental diseases (Kushiyama *et al*. 2009; Feghali *et al*. 2012). These polyphenolic compounds prevent biofilm formation by *P. gingivalis* and *Fusobacterium nucleatum* and reduce the activity of several *P. gingivalis* proteases. Epicatechin-3-O-gallate-(4β,8)-epicatechin-3′-O-gallate isolated from the aerial parts of *Rumexacetosa* L. interacts with the active side of Arg-gingipain and hemagglutinin from *P. gingivalis* (Schmuch *et al*. 2015). Furthermore, the antimicrobial activity of flavogalonic acid dilactone and terchebulin from a methanolic extract of *C. hartmannianum* that inhibited metalloproteinase-9 matrix of *P. gingivalis* growth (Mohieldin *et al*. 2017).

The non-specific inhibition of HTs might be connected to their ability to neutralize free radicals (–R) by donating a hydrogen atom (–RH) or an electron (–R–), chelating metal ions in aqueous solutions, and binding or precipitation of proteins due to extensive coating of hydrophobic surfaces of peptides. To identify the real reasons and inhibition mechanism, more investigation is required of the chemical formula of HTs (Smith *et al*. 2005; Aaby *et al*. 2007).

Conclusion

The water extracts of the selected plants can play a potent role in blocking the very early stages of *P. gingvalis* virulence-related factors. Thus, targeting those virulent factors might hinder pathogenicity and improve the expansion of new, safe, effective therapeutic and/or preventative agents. It is a challenging and complicated task to impede all of the virulence-related factors (Ingar and Potempa 2014). The HTs from *O. vulgare* leaves show a wide spectrum, impeding most of the targeted virulence-related factors in this study. The polarity and water solubility of this compound might be suitable for further applications to support oral hygiene.

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