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ANTIVIRAL AND ANTI-QUORUM SENSING ACTIVTIES OF LYOPHILIZED AQUEOUS EXTRACT OF PROPOLIS FROM SÉTIF

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ABSTRACT

Aqueous extracts are preferentially used in traditional medicine in Algeria. Among these extracts, propolis is used for the treatment of wounds, boils, canker sores, burns. The purpose of this study was to evaluate the antiviral/antiquorum sensing (anti-QS) characteristics of Propolis aqueous extract collected from bee hive near Sétif (east of Algeria) against human pathogenic viruses and gram negative bacteria. First the Maximal Allowable Concentration (MAC) of the lyophilized aqueous extract of propolis was determined to avoid the toxicity of the extract. A human lymphoid cell line that contains Epstein Barr Virus (EBV) genome as an episome (P3HR-1) and HEp-2 infected with Coxsackievirus (CVB4), were selected according to their ability to deliver endemic infectious viruses at high titers. Antibacterial activity was screened by evaluating anti-QS capacity of the extract. Pre-treatment revealed that the MAC of the extract reduces the viral titer of Coxsackievirus by half a log from $TCID_{50} = 10^{5.07}$ to $TCID_{50} = 10^{4.6}$, and protected HEp-2 against CVB4 infection. While no antiviral effect on the EBV replication was obtained. The anti-QS capability of the extract was showed against *Chromobacterium violaceum* strain 026, and by protecting *Artemia* from *Vibrio harveyi* BB120 infection.The aqueous extract pre-treatment has not antiviral protection, for DNA viruses. It protected HEp-2 cells against CVB4 infection, degraded Acyl Homoserines lactones (AHL), and protected animal *Artemia*.

Keywords: lyophilized aqueous extract; propolis; anti-QS; antiviral; cytotoxicity

INTRODUCTION

In big cities like Algiers, there are herbalists essentially at the level of the markets, and their stalls are frequented by a broad public which goes from the adept diligent, convinced of the benefits of the alternative medicines, to the indigent patient in search of an accessible treatment **(Hammiche *et al.*, 2013)**. Hive products have been used by humans for millennia. Beehive products such as honey, propolis, and royal jelly were extensively used to treat several diseases **(Pasupuleti *et al.*, 2017)**. Propolis is still the most used one in folk medicine worldwide. It is collected from sap flows, bark, and leaf buds of a considerable variety of plants, ranging from annual plants to perennial ones. However, the most plants visited for such collection are poplar and conifer trees **(Nadjafi *et al.*, 2007)**. Recent studies revealed a new type that was named Mediterranean Propolis, which contains high levels of diterpenoids. This propolis was collected from Greece **(Popova *et al.*, 2010; Celemli *et al.*, 2013)**, Malta **(Popova *et al.*, 2011)**, Turkey **(Silici *et al.*, 2007; Duran *et al.*, 2011)**, Algeria **(Piccinelli *et al.*, 2013; Soltani *et al.*, 2017; Chaa *et al.*, 2021).** Bees use propolis as an immunity component that protects their community from micro and macroorganisms' invasion. The propolis is gifted by its anti putrefaction activity against animal corpses. **Rufatto *et al.* (2017)** confirmed that propolis has many activities, antimicrobial, anticancer, and antioxidant activities. These activities are directly related to; its chemical components, the variability of flora and harvest time, bee species as well as the processing technique **(Calegari *et al.*, 2017; Rufatto *et al.*, 2017)**. During the two last decades, several studies have explored the capabilities of the aqueous extract of propolis. These reported several activities as; antitumor either against malignant transformation by retroviruses **(Huleihel and Ishano, 2001)** or inhibition of malignant cell lines **(Nadjafi *et al.*, 2007)**, antioxidant **(Gülçin *et al.*, 2010)**, immunomodulatory **(Orsatti et al., 2010; Soltani et al., 2017)**, protective against Ultraviolet A **(Butnariu and Giuchici, 2011)**, antiviral **(Bufalo *et al.*, 2009)**, anticariogenic **(Oršolić *et al.*, 2003)** and antimicrobial **(Domacoski *et al.*, 2010; Monte *et al.*, 2014; Soltani *et al.*, 2017, 2021)**. Plant extracts may have activities that inhibit bacterial virulence and pathogenicity. These effects are neither bactericidal nor bacteriostatic and do not put pressure to develop resistance **(Chenia, 2013)**.

In an editorial **Williams (2006)** reported that virulence factors constitute one of a variety of bacterial physiological processes regulated via ''Quorum Sensing''. Antimicrobial chemotherapy aimed at inhibiting the regulation of virulence factor expression could have a more global effect on the ability of an organism to establish infection **(Alksne and Projan, 2000)**. Powerful antipathogenic compounds able to target bacterial signaling systems are present in nature. These compounds **(Hentzer and Givskov, 2003)** such as Cinnamaldehyde **(Brackman *et al.*, 2011)** interfere directly with Quorum sensing (QS) signaling systems that control biofilm formation, pathogenicity, and virulence, which brings an attractive target for developing drugs that control microbial activity **(Hentzer and Givskov, 2003)**. In the present study two goals were targeted: (i) The first goal evaluated the cytotoxic/protective power of the lyophilized aqueous extract of Propolis in the presence and absence of virus or bacteria; and (ii) the second goal focused on the investigation of an eventual mode of activity of the extract: its anti quorum sensing (anti-QS) power.

MATERIAL AND METHODS

Material

Propolis

The raw propolis was harvested by scraping the hive honeycombs on a site (El Guasria) adjacent to the central campus of the Ferhat Abbas University Sétif 1 between August and September 2012.

Bacterial Strains

Chromobacterium violaceum strain 026, *Vibrio harveyi* BB120, and *Artemia* cysts were from the laboratory of Dr. Natrah FMI Faculty of Agriculture, University Putra Malaysia.

Cell lines and viruses

Human lymphoid cell line (P3HR-1 cells) that contains Epstein Barr Virus (EBV) genome as an episome,HEp-2 cells derived from human squamous cell carcinoma of the larynx and Coxsackievirus (CVB4); have been provided respectively by Viral Oncogenesis Laboratory and the referenced Enterovirus Laboratory for the Coxsackievirus (Pasteur Institute of Algiers, Algeria). The CVB4 virus was propagated in HEp-2 cells in Eagles Minimum Medium (EMEM) enriched with 2% fetal calf serum (FCS) and stored at -80°C.

Methods

Lyophilized Aqueous Extract

The lyophilized aqueous extract of propolis was obtained as previously described by **Haichour *et al.* (2021)**. Extracts solutions were prepared by dissolving 100 mg/mL of the lyophilized powder obtained in sterile water, filtered through Millipore millex syringe driven filter unit (0.22µm). Then a serial dilution (1/2 and 1/4) of each solution is made for the anti-QS, and for antiviral activity, 25mg/mL were used in a serial dilution by half till 7.81µg/mL.

Cell culture

Cells of P3HR-1, were cultivated at a rate of 5.10^5 cells/mL in Roswell Park Memorial Institute (RPMI1640) medium supplemented with 10% FCS from Welgene **(Hinuma *et al.*, 1967)**, 1% of antibiotic (Penicillin-streptomycin; Sigma-Aldrich®), and1% of the L-Glutamine (Sigma-Aldrich®); incubated at 37°C with 95% of humidity and 5% CO_2 **(Zur Hausen *et al.*, 1979)**.
Cells of HEp-2 were cultivated in Hank Minimum Essential Medium (HMEM) supplemented with 10% FCS, 1% L-Glutamine, and 100 µU/mL of Penicillin and 100 µU/mL of Gentamicin (Sigma-Aldrich®) **(Gorphe, 2019)**.

Cytotoxicity test of the extract

Cytotoxicity of the extracts was performed according to **Abid *et al.* (2012)** with some modifications, instead of MTT (3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) crystal purple for HEp2 and trypan blue for P3HR-1 cells which were reduced by viable cells **(Strober, 2015)** and. The viability of the cells was evaluated spectrophotometrically at 492 nm by the Crystal Purple (0.5%) staining method for HEp2 **(Ait-Mbarek *et al.*, 2007)**, and Trypan blue method for P3HR-1**(Strober, 2015)**. Cell suspensions (5×10^5 cells/mL) were distributed (100 µL/well) and cultivated as described in cell culture section using 96-well plates, and after incubation at 37°C for 24h they were treated with 50 µL of the extract at different concentrations, the cells were incubated for an additional time of 48 h **(Abid *et al.*, 2012)**. The blank consist of wells receiving cells without treatment. The cytotoxicity percentage in HEp2 was calculated as [(U - T) / U] x 100, where U and T were respectively the OD492 of untreated and of treated cells. The 50% cytotoxic concentration (CC50) calculated by regression analysis was defined as the compound's concentration (mg/mL) required for the reduction of cell viability by 50%. While for P3HR-1 cells the percentage of cell viability (Vi) was performed by inverse microscopy enumeration using the following formula **(Anonymous, 2006)**: Vi %= number of Clear cells/Total Cells number X 100.

Viruses' inhibition assays

Confluent HEp-2 cells in microplates, were treated with decreasing concentrations of the extract from the MAC (Maximal Allowable Concentration: nontoxic) between 0.78 mg/mL and 0.09 mg/mL. After 2 hours of incubation; 100 µl $TCID_{50}$ (Tissue Culture Infectious Dose at 50%) of the viral suspension were added and incubated for 48 hours at 37°C under 5% CO_2. Viral inhibition effect (cell viability) and cytopathological change were assessed using values of Crystal violet absorbance at 492 nm after 24 and 48 h. Inhibitory concentration 50% (IC_{50}) corresponds to the concentration which decreases by 50% the cytopathic effect of the virus (50% cellular protection). The protection percentage was calculated according to the following formula:
Percent protection = [(ODT) V - (ODC) V] / [(ODC) M - (ODC) V] ×100. Where (ODT) V, (ODC) V, and (ODC) M indicate respectively; absorbance of the treatment, the virus-infected control, and mock-infected control **(Abid *et al.*, 2012)**.
Cells of P3HR-1 were cultivated as in the precedent test, the Falcon flasks were incubated for 3h at 37°C. In each Falcon flask except the cell control, TPA (Tetradecanoyl-phorbol-13-acetate) was added at a final concentration of 20ng / mL (TPA is dissolved in Dimethyl Sulfoxide (DMSO) at one mg/mL). Then flasks were incubated for 72h as previous conditions. Antiviral activity was performed by Trypan blue method or by Indirect Immune Fluorescence (IFI) **(Strober, 2015)**.

Screening the anti-QS

Chromobacterium violaceum strain 026 (CV026) was grown in Luria Bertani broth (LB) with 20 µg/mL Kanamycin (SIGMA) and 5 µg/mL of N-Hexanoyl-DL-Homoserine Lactone (SIGMA) for 48 h at a temperature of 32°C with 140 rpm aerobically to an estimated concentration of 10^9 cell/mL. 20 mL of CV026 were mixed with 80 mL soft LB Agar (0.8%, SIGMA) and homogenized. The mixture was poured onto hard Agar (1.5%) to have a double layer, after its solidification, wells were made in the upper layer using a sterile Pasteur pipette. 20 µL of the prepared extract of each dilution was filled in the prepared well. Negative controls consist of distilled water; the positive control was Trans-cinnamaldehyde (SIGMA Aldrich) at 0.1M. Plates were incubated overnight at 32°C, CV026 was used to detect and respond to the presence of Acyl Homoserines lactones (AHL) molecules through the synthesis of purple pigmentation (violacein), absence of the purple color indicated inhibition of violacein and degradation of AHL molecules (degradation of QS activity). The diameters of the non pigmented observed zones were measured **(Noorashikin *et al.*, 2016)**. To confirm the degradation of AHL another assay was performed as follows.

AHL degradation assay

For this purpose, 10 µg/mL of N-Hexanoyl-DL-Homoserine Lactone (HHL, 8µl/2mL) were added to the positive control as well as the extract solution. This later was filtered through a 22µm filter and then 10 µL were spotted on the LB agar plate already inoculated with 100µL of CV026. The last step was repeated after 4 hours **(Noorashikin *et al.*, 2016)**. The QS degradation activity was observed through violacein inhibition, and the diameters of the non pigmented observed zones were measured.

Artemia Challenge

Artemia was used for testing the toxicity of the extract on living organisms, according to **Soto–Rodriguez *et al.* (2003)** with some modifications. First cysts of *Artemia* were decapsulated aseptically in sterile seawater to facilitate their hatching. Decapsulated cysts in falcon tubes were placed on aerator with constant light at 28°C, and incubated for 24h at least. Secondly, two batches of *Artemia* with 12.5µg/mL of the extract were prepared, one for testing its toxicity and the second for protecting *Artemia* against *V. harveyi* BB120 (10^6cell/mL); in addition to the control batches consisting of *Artemia* (i) without extract and bacteria (-ve one) (ii) and without extract but with bacteria (+ve one). The batches consist of 20mL of sterile seawater to which 100µL of yeast extract (10g/L) and twenty hatching *Artemia* were added. The experiment was repeated two times with two repetitions.

Statistical analysis

Data were analyzed using Graph Pad Prism 8.4.2 Statistical software (Graph Pad Software, USA). Analysis of Variance (ANOVA), one way and Tukey's (anti Qs, Hep2 protection); two way and Tukey's (Artemia viability, P3HR-1, and Hep 2 cell cytotoxicity), two way and Sidak's multiple comparisons test (AHL degradation). Data were presented as mean ± standard error (SEM) and differences were considered significant at $P < 0.05$.

RESULTS

Cytotoxicity and antiviral effect of lyophilized aqueous extracts

The lyophilized aqueous extract of propolis showed a cytotoxic effect on the viability of tested cells (HEp-2, fig. 1 and P3HR1, fig. 2) with a CC 50 at 7 mg/mL for HEp-2 cells and 0.230 mg/mL for P3HR1 cells.
While concentrations of 0.78, 0.39, and 0.19 mg/mL; showed no inhibition effect on HEp-2 cells (100% of the cells are viable); the same was observed with P3HR1 at concentrations bellow 125µg/mL. Concentrations of 25, 12.5, 6.25, 3.12, 1.56 mg/mL, and 500, 250 µg/mL acted respectively on the growth and appearance of HEp-2 and P3HR1 cells with different inhibition percentages compared to untreated control. A concentration of 25 mg/mL inhibited 60% of HEp-2 cells; at 12.5, 6.25, 3.12, and 1.56 mg/mL, the inhibition percentage was respectively about 54, 49, 40 and 28% as shown by the Purple Crystal method; and P3HR1 cells were inhibited at a rate of 67.96% when treated with 500µg/mL shown by Trypan blue method.
By evaluating its antiviral effect and compared to positive control, the MAC of the extract reduces the viral titer of Coxsackievirus by half a log from $TCID_{50} = 10^{5.07}$ to $TCID_{50} = 10^{4.6}$. Concerning EBV and after induction of P3HR1cells by TPA, the results obtained showed no antiviral effect on the replication of the virus, the expression of the Viral Capsid Antigens (VCA) by IFI was very significant.
Otherwise, the aqueous extract of propolis showed a protective effect against CVB4 infection (fig.3), it inhibited the multiplication of the virus in HEp-2 cells, with an IC_{50} of 0.53 mg/mL and a selective (therapeutic) index (SI) of 13.20. A

total destruction of the cellular layer was obtained with concentrations of 0.19, 0.09, 0.04, 0.02, 0.01 and 0.006 mg/mL; while concentrations of 0.78 and 0.39 mg/mL showed a protective effect against CVB4 infection, with protection percentages of 76% and 37% respectively.

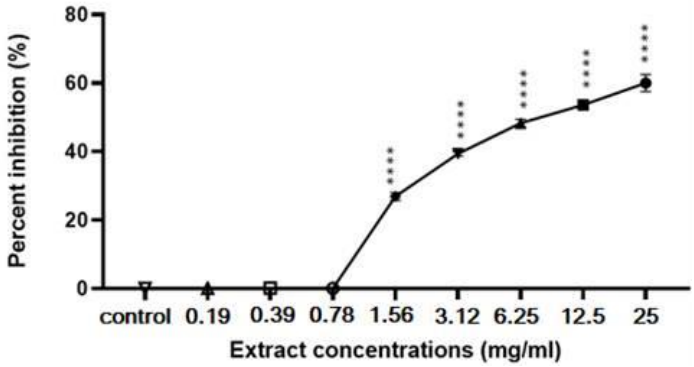

Figure 1 Cytotoxicity of the lyophilized aqueous extract of Propolis on Hep2 cells ****: Statistically very significant (P<0.0001)

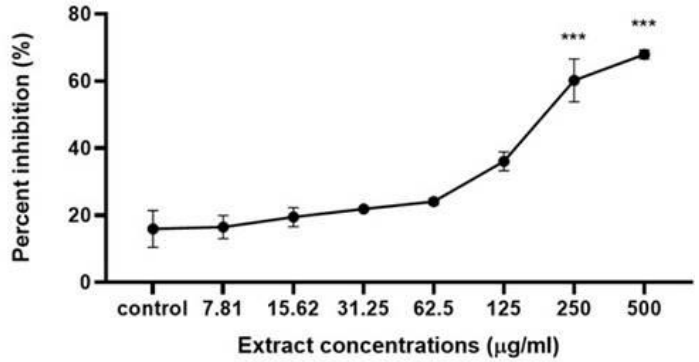

Figure 2 Cytotoxicity of the lyophilized aqueous extract of Propolis on P3HR1 cells. ***Statistically significant (P<0.0004)

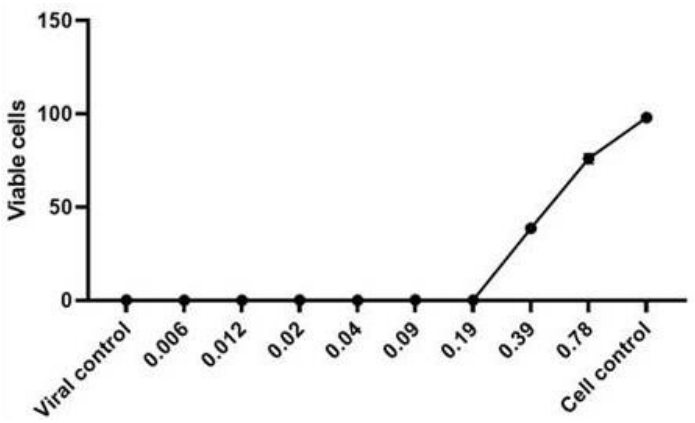

Figure 3 Protection of Hep2 cells against infection by CVB4

Antibacterial activity versus Anti-QS activity

Artemia Challenge

In the *in vivo* virulence test *Artemia* survived differently when it was infected with *Vibrio harveyi* BB120 in the presence and absence of the extract; amongst twenty *Artemia* used in the first batch 19 ±1 were still alive, they were grown and moved well after 24h of feeding. While in the second batch treated with propolis extract and inoculated with *V. harveyi* BB120, 77.6% of the organisms were still alive and being well after 24h of feeding too. On the other hand, the positive control with *V. harveyi* BB120 decreased to 12 ±1 organisms (60% alive) after 6 h of treatment only (fig. 4).

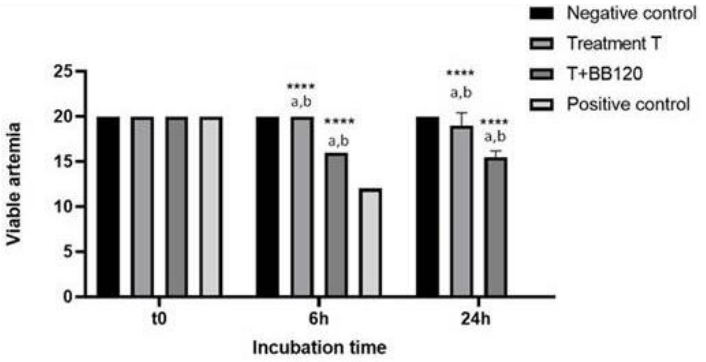

Figure 4 Protection of *Artemia* against infection by *Vibrio harveyi* BB120
Negative control: Neither treatment nor BB120; T: treatment with aqueous extract at 12.5µg/mL; T+BB120: treatment with aqueous extract at 12.5µg/mLl+ *Vibrio harveyi* BB120 at 10^6cell/mL; positive control: *Vibrio harveyi* BB120 at 10^6cell/mL; ****: Statistically very significant (P<0.0001)

Anti-QS activity of the lyophilized aqueous extract

To understand partially how the extract protected *Artemia* against *V. harveyi* BB120, its anti-QS capacity was evaluated. As compared to a positive control consisting of Cinnamaldehyde, the inhibition, by the extract, of QS was demonstrated by the loss of the purple pigment in strain 026 of *Chromobacterium violaceum*, thus showing a clear zone around the wells and the diameter of which was concentration-dependent. The negative control was only water which remained purple (fig. 5, 6).

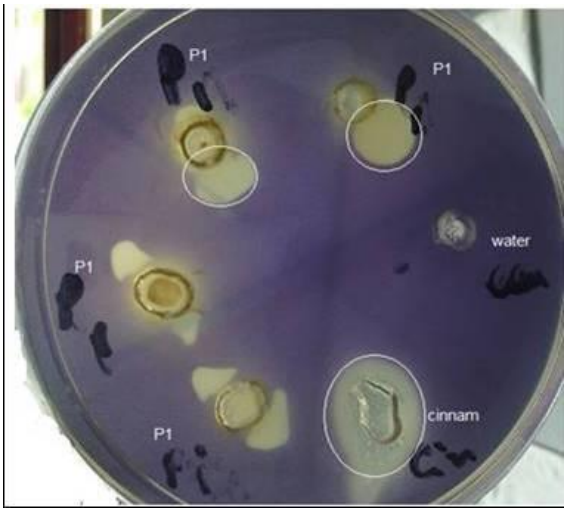

Figure 5 AntiQuorum Sensing screening
P1: lyophilized aqueous extract of propolis at 100mg/mL, water: negative control, cinnam: positive control (cinnamaldehyde at 0.1M)

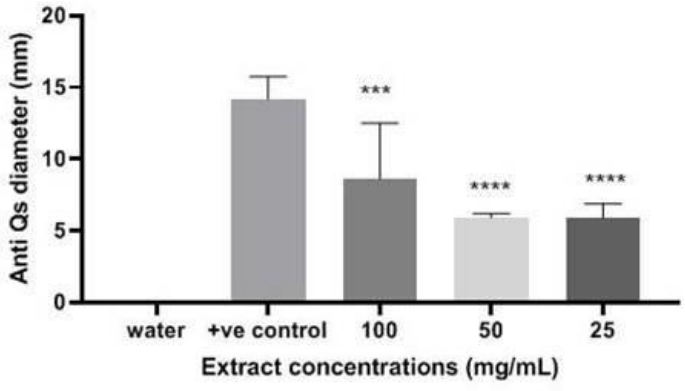

Figure 6 AntiQuorum Sensing activity of Propolis lyophilized aqueous extract
100, 50 and 25mg: aqueous extract of propolis diluted/ml, water: negative control, cinnam: positive control (cinnamaldehyde at 0.1M); Anti-Qs diameter: diameter of the degradation zone of the quorum sensing compound produced by *Chromobacterium violaceum* strain 026. ****: Statistically very significant (P<0.0001), ***: Statistically significant (P<0.0004)

Degradation of QS molecules

The degradation power of the extract (fig.7) was confirmed using the agar diffusion method, by the absence of pigmentation (inhibition of the violacein purple color regulated by QS) in the inoculated zone. This latter was increased after 72h (fig.8) as compared to positive and negative controls. The extract inhibited the pigmentation with mean a diameter of 12.05±1.11 mm, while the positive control inhibited with a mean diameter of 14.20±1.42 mm.
The statistical analysis revealed that no significant differences were obtained for all the antiviral tests, while a significant difference was obtained for AHL degradation one.

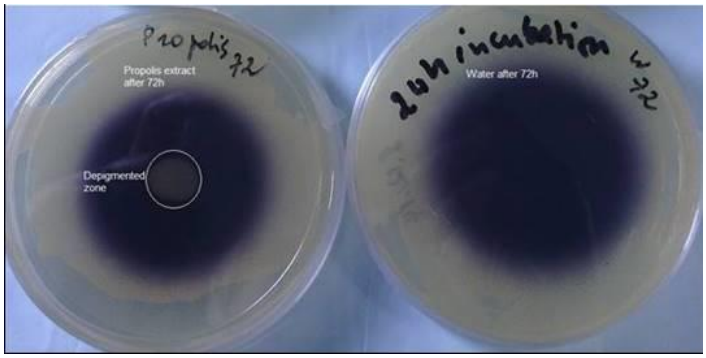

Figure 7 Degradation of Acyl Homoserine Lactone by lyophilized aqueous extract of Propolis
On the left: degradation zone (depigmentation) after 72h of incubation, on the right: negative control inoculated with water after 72h.

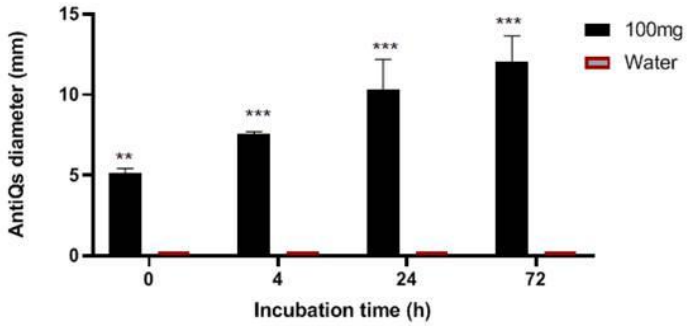

Figure 8 Gradually increasing of the degradation zone
Anti-Qs diameter: diameter of the degradation zone of the quorum sensing compound (Acyl homoserine lactone); ***: Statistically significant (P<0.001), **: Statistically less significant (P<0.002)

DISCUSSION

In Algerian traditional medicine, plant preparations are most often aqueous and sometimes based on olive oil. That's why the purpose of this study was to test cytotoxic activity of the lyophilized aqueous extract and secondly determining its effectiveness against pathogens.
In the present study, the lyophilized aqueous extract of propolis showed a cytotoxic effect on the growth of HEp-2 and P3HR1cell lines, where 54% of the HEp-2 cells were inhibited by 12.5mg / mL, and 50% of the P3HR1 cells were inhibited by 190 µg/mL. Similar results were obtained by **Nadjafi *et al.* (2007)**, while there are differences in the propolis extract concentrations, the propolis extracts were toxic to McCoy, BHK21, HEp-2, and HeLa; with inhibition up to 75% of HEp-2 cells at a concentration of 2 mg/mL. They reported that treatment of the cells by aqueous extract of propolis acted as antiproliferative cells, it helps to kill the proliferative cells and stimulate normal cells multiplication during the treatment. In contrast, in another study, there was no effect of the lyophilized propolis aqueous extract on cell viability (similar to the mock) of head-kidney leucocytes from gilthead seabream (*Sparus aurata* L.) when it was used at 100 and 200µg/mL **(Soltani *et al.*, 2017)**.
On the other hand at non cytotoxic concentrations, the extract inhibited Coxsackievirus (CVB4) replication in HEp 2 cells and protected them against CVB4 infection, while VCA was expressed in P3HR1 cells; before or simultaneously to virus infection. **Bufalo *et al.* (2009)** demonstrated that the best antiviral activity against poliovirus 1(PV1) replicated in HEp-2 cells was obtained in the simultaneous administration of propolis, as the same time the viral quantification was relatively lower. The propolis extract tested would have partially blocked the penetration and entry of viral particles into the cytoplasm of infected cells; this step is necessary for the virus to continue its replication **(Huleihel and Ishano, 2001; Bufalo *et al.*, 2009)**, or lead to RNA degradation before the virus entry into cells or after their release to the supernatant **(Bufalo *et al.*, 2009)**.
In addition to the cytotoxic effect against the growth of lineage cells (HEp-2 and P3HR1), the expression of surface antigens (VCA) of the EBV rather than the proteins involved in tumor genesis would be attributable to an anti-proliferative effect. Pretreatment and continuous treatment of propolis extract in NIH/3T3 cell cultures showed an impressive inhibition of malignant cell transformation by Moloney murine Sarcoma Virus (MuSV) **(Huleihel and Ishano, 2001)**.
The antiviral and the anti proliferative activities of the extract of propolis could be attributed to its contents in flavonoids (3,047 ± 0,004 mg/g), polyphenols (221 ± 0,001 mg/g), and its four identified derivatives of cinnamic acid **(Soltani, 2017; Soltani *et al.*, 2021)**.
Cinnamaldehyde a derivative of cinnamic acid has been reported as an inducer of apoptosis on various cancer cell lines *in vitro*, that transduced the apoptotic signal *via* reactive oxygen species (ROS) generation **(Ka *et al.*, 2003; Li *et al.*, 2016)**. Investigating flavonoids from plant extracts against Coxsackievirus B3 (CVB3), **Abid *et al.* (2012)** demonstrated that flavonoids enhanced antiviral activity at noncytotoxic concentrations. While earlier, **Bufalo *et al.* (2009)** demonstrated that cinnamic acid a compound from propolis has also an antiviral activity against replication of PV1. **Hazam *et al.* (2017)** demonstrated that direct contact with acyclovir and other pure molecules e.g. quercetin, caffeic acid and chlorogenic acid were very effective in inhibiting Herpes simplex 1 virus (HSV1) and varicella zoster virus (VZV) while in the case of adenovirus type 5 (ADV5) most of substances were not so efficient. The propolis aqueous extract was most effective in pre-treatment of ADV5 – infected cells, more efficient than the propolis tincture, as well as more efficient than acyclovir; while post-treatment it did not protect HSV1 infected cells **(Hazam *et al.*, 2017)**. These findings may explain partially the non activity of the lyophilized aqueous extract on EBV (DNA virus) and *vice versa*.
The inhibition of cell growth, necrosis, apoptosis **(Oršolić *et al.*, 2001, 2003)**, and metastasis formation **(Oršolić and Bašić, 2003, 2005a)** in tumor cells are the main mechanisms by which propolis acted. Metastasis is mediated by immunomodulatory activity by increasing macrophages activity **(Oršolić and Bašić, 2003)**.
Using continuous and discontinuous drug exposure methods on P3HR1cell lines in an unpublished data, **Rihane and Ouanes (2017)** showed a dose dependent apoptotic effect and DNA fragmentation of the lyophilized aqueous extract of propolis tested in the present study. The anti metastatic activity as well as its antitumor activity **(Oršolić *et al.*, 2005)**, were the result of synergistic activities of a water-soluble derivative of propolis (WSDP) components **(Oršolić and Bašić, 2005b)** as flavonoid e.g. caffeic acid a cinnamic acid derivate **(Oršolić et al., 2001)** and polyphenolic compounds **(Oršolić *et al.*, 2003)**.
The lyophilized aqueous extract of propolis protected *Artemia* against bacterial infection and inhibited the QS activity. This extract has proved bactericidal activities against several bacteria; *Vibrio harveyi, Photobacterium damselae* **(Soltani *et al.*, 2017)**, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC10876, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumoniae* ATCC700603 and *Enterobacter cloacae* **(Soltani *et al.*, 2021)**, respectively two marine opportunistic **(Soltani *et al.*, 2017)** and human pathogenic bacteria **(Soltani *et al.*, 2021)**. The lowest bactericidal activity was found against *P. damselae* **(Soltani *et al.*, 2017),** *P. aeruginosa* ATCC27853, *Klebsiella pneumoniae* ATCC700603 and *Enterobacter cloacae* **(Soltani *et al.*, 2021)**; while the highest was in the case of *V. harveyi* **(Soltani *et al.*, 2017)** *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC10876 **(Soltani *et al.*, 2021)**. **Monte *et al.* (2014)** have already demonstrated that ferulic acid (hydroxycinnamic acid) was more effective than gallic acid (hydroxybenzoic acid) against *Escherichia coli* and *Staphloccocus aureus*.
In the presence of *Vibrio harveyi* BB120, with and without the addition of the extract *Artemia* survives differently. The extract significantly increased the survival of *Artemia* after infection. Earlier, **Brackman *et al.* (2011)** reported the increased survival of *Caenorhabditis elegans* nematodes infected with *V. harveyi, V. anguillarum* and *V. vulnificus* in the presence of cinnamic acid and its structural analogs.
These activities can be assigned to its components as, benzoic acid, cinnamate, and its derivatives which were present in the extract **(Soltani *et al.*, 2017)**. Cinnamic acid derivative, cinnamaldehyde, and most analogs reduced the *Vibrio* species starvation response, affected biofilm formation in *V. anguillarum*, *V. vulnificus*, and *V. cholera*, protease production in *V. anguillarum* and *V. cholera*, and pigment production in *V. anguillarum* . They blocked at least AI-2 (autoinducer-2) QS **(Brackman *et al.*, 2011)**.
Our data showed decreased violacein production when the extract was added exogenously, as observed through halo zone formation, as obtained by **Kalia *et al.* (2015)**. As expected, zones of QS inhibition were also observed with the control cinnamaldehyde. This latter has been reported as HSL degrader **(Noorashikin *et al.*, 2016)**.
Finally, the aqueous propolis extract, had antiviral activity against CVB 4, do not provide antiviral protection in the case of EBV, and had an anti-QS activity which correlated with the QSI (quorum sensing inhibition) activity that disrupts QS AHL bacterial communication mechanism. On the base of these results, more investigations are needed for the study of the Algerian propolis extract.

CONCLUSION

The use of natural compounds to attenuate viral and bacterial pathogenicity is an attractive approach, particularly if, at the dosages used these inhibitors are nontoxic for living organisms. In this study, the aqueous extract of propolis from Sétif possess anti viral activity against B4 Coxsackievirus and an anti-QS effect in *C. violaceum*. The use of the extract as quorum sensing-disrupting compound protects living organisms as *Artemia* larvae from *V. harveyi* BB120 without a negative effect on the growth of the larvae.

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