The evaluation of the cytotoxic effect and the antibacterial, antifungal, and antiviral activities of *Hypericum triquetrifolium* Turra essential oils from Tunisia

ROUIS Zyed\textsuperscript{a,}\textsuperscript{*}, KOUDJA Sadok\textsuperscript{b}, Yangui Thabet\textsuperscript{c}, ABID Nabil\textsuperscript{a}, CIONI Pier Luigi\textsuperscript{d}, FLAMINI Guido\textsuperscript{d}, AOUNI Mahjoub\textsuperscript{a}

\textsuperscript{a}Laboratoire des Maladies Transmissibles et Substances Biologiquement Actives LR99ES27, Faculté de Pharmacie de Monastir, Université de Monastir, Tunisia.

\textsuperscript{b}Laboratoire d’Analyse, Traitement et Valorisation des Polluants de l’Environnement et des Produits, Faculté de Pharmacie Rue Avicenne, Monastir 5000, Tunisia.

\textsuperscript{c}Laboratoire des Bioprocédés, Pôle d’Excellence Régionale AUF, (PER-LBP) Centre de Biotechnologie de Sfax, BP: 1177, 3018 Sfax, Tunisia.

\textsuperscript{d}Dipartimento di Chimica Bioorganica e Biofarmacia, Universita` di Pisa, Via Bonanno 33, 56126 Pisa, Italy

ROUIS Zyed\textsuperscript{*} (zyedrou@yahoo.fr), KOUDJA Sadok (khouadja_sadok@yahoo.fr), Yangui Thabet (thabette_yangui@hotmail.com), ABID Nabil (nabilabid@udbukltd.com), CIONI Pier Luigi (cioni@farm.unipi.it), FLAMINI Guido (flamini@farm.unipi.it), AOUNI Mahjoub (aouni_mahjoub2005@yahoo.fr)

\textsuperscript{*}Corresponding author: ROUIS Z (phone: +216-97-568817; fax: +216-73-465754; e-mail: zyedrou@yahoo.fr. Laboratory of Transmissible Diseases and Biological active Substances, Faculty of Pharmacy, Avenue Avicenne, 5000, Monastir, University of Monastir, Tunisia)

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Abstract

Background: A number of bioactive secondary metabolites have been identified and reported for several Hypericum species. Many studies have reported the potential use of the plant extracts and their essential oils against several pathogens. However Hypericum triquetrifolium is one of less studied species for their antimicrobial activities. The aim of the present study was to evaluate the cytotoxic effect of Hypericum triquetrifolium essential oils as well as their antimicrobial potential against coxsakievirus and a range of bacterial and fungal strains.

Methods: The essential oils of Hypericum triquetrifolium harvested from five different Tunisian localities (Fondouk DJedid, Bou Arada, Bahra, Fernana and Dhrea Ben Jouder) were evaluated for their antimicrobial activities by microbroth dilution methods against bacterial and fungal strains. In addition, the cytotoxic effect and the antiviral activity of the tested oils were carried out using vero cell lines and coxsakievirus B3.

Results: The results of tests carried in the present work showed potent antibacterial activity against a wide range of bacterial strains with MICs = 0.39 – 12.5 mg/mL; MBCs = 1.56 – 25 mg/mL). In addition, the tested essential oils showed promising antifungal activity with MICs values 0.39 – 12.5 µg/mL, and MFCs ranged between 3.125 and 25 µg/mL and significant anticandidal activity with MICs 0.39 – 12.5 µg/mL, and MFCs ranged between 3.125 and 25 µg/mL. Although the tested essential oils showed low cytotoxic effect (CC50 range: 0.58 – 12 mg/mL), they did not show antiviral activity against coxsakievirus B3.

Conclusion: The tested essential oils from Hypericum triquetrifolium can be used as antimicrobial agents and could be safe at non cytotoxic concentrations. As shown for the
tested essential oils, comparative analysis need to be undertaken to better characterize the antimicrobial activities of *Hypericum triquetrifolium* extracts with different solvents as well as the purified fractions and their pure molecules.

**Keywords:** *Hypericum triquetrifolium*; Coxsakievirus B3; Essential oils; Bacteria; Fungi.

**Background**

Essential oils are aromatic extracts which have been used for a long time as flavouring agents and constituents of several commercial products. Chemical composition of the essential oil varied from one plant part to another. In addition, this chemical composition of the essential oils may differ according to the site of collect (geographical provenence), as their components play a major role in the plant adaptation to its ecology and environment behaviors including biotic and abiotic factors [1, 2]. Currently, the importance of the use of essential oils is more highlighted due to their increasing demand for food, cosmetics and pharmaceutical industries. In addition, the interest in essential oils has increased as potential alternatives for therapeutic purposes against common microbes. Bacterial resistance is spreading throughout the world primarily due to excessive use of antibiotics and poor infection control practices in hospitals, making it one of our times biggest issues. The scientific literature revealed the antimicrobial, antifungal and antioxidant potential of several essential oils [3, 4]. In addition, the antiviral potential of essential oils was well documented [5, 6].

Microorganisms such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Enterococcus faecalis* (*E. faecalis*), *Pseudomonas*
*P. aeruginosa*, and *Escherichia coli* (*E. coli*) are frequently isolated from skin wounds in humans and animals. In addition, *S. epidermidis* infections are commonly acquired in the hospital as a result of contamination of surgical cuts with microorganisms from the patients themselves or from the hospital personnel [7]. Infection with *P. aeruginosa* is the most serious complication in burns patients [8, 9], followed by infections with *E. coli*, *S. aureus* and other microorganisms [8]. Infection with *Bacillus cereus* has been well documented in the literature for over a century which is generally associated with gastroenteritis caused by the consumption of infected aliments. *Vibrio alginolyticus* is ubiquitous in seawater and tends to cause superficial wound and ear infections (otitis media and otitis externa) [10]; this infection can progress to bacteraemia and necrotising fasciitis, particularly in the immunocompromised patients [11]. *Vibrio cholerae* (*V. cholerae*), a Gram (−) bacterium and the causative agent of cholera, has caused seven pandemics since 1816, as well as sporadic inter-epidemic outbreaks. *V. cholerae* is autochthonous in a region of the world where cholera never occurs and that the human body is not an obligate environment for the presence and dispersal of this organism [12]. *Salmonella typhimurium* causes typhoid fever associated with gastroenteritis. The infection is caused by consuming contaminated food or drinks. *Aeromonas hydrophila* (*A. hydrophila*) has been receiving increasing attention both as an opportunistic and as a primary pathogen of both humans and aquatic and terrestrial animals [13]. *A. hydrophila* inhabits aquatic environments and the gastrointestinal tract of healthy fish. It also commonly occurs in foods, milk, red meats and poultry [14-16]. It causes disease and mortality mainly in freshwater fish but sometimes in marine fish [16]. The bacterium also infects humans and causes lesions ranging from gastroenteritis to septicaemia [17].
The genus *Hypericum* is a member of the *Hypericaceae* family [18, 19]. A number of bioactive secondary metabolites have been identified and reported for several *Hypericum* species [20-22]. Essential oils extracted from *Hypericum* species are well documented for their antimicrobial activities [3, 23-32].

*Hypericum triquetrifolium* Turra (*H. triquetrifolium*), native to Eastern Europe and the Mediterranean area, has been used traditionally for its sedative, antihelminthic, anti-inflammatory, and antiseptic effects [23, 33]. In addition, several studies have reported the potential use of the essential oil and crud extracts of *H. triquetrifolium* as therapeutic substances, mainly in the treatment of burns, gastroenteritis, as well as its effectiveness antinociceptive, and antioxidant drugs [34-36]. However, *H. triquetrifolium* is one of less studied species for their antimicrobial activities. According to the literature data reporting the antibacterial activity of *H. triquetrifolium*, a study has reported the evaluation of the plant essential oil by studying the zone of growth inhibition against a number of bacterial and candida strains [37].

In the present study, the antimicrobial activities of essential oils extracted from *H. triquetrifolium*, were analysed *in vitro*. The variation in their activities was discussed according to their chemical compositions reported previously [38].

**Material and methods**

**Plant material and essential oil extraction**

Voucher specimens identified by Prof. Mohammed El Hedi El Ouni (Department of Biology, Faculty of Sciences of Bizerte, Tunisia) and have been deposedited in the Herbarium of the Laboratory of Transmissible Diseases and Biological Active
Substances (Faculty of Pharmacy of Monastir, Tunisia), under the following accession codes: *H. tri.* 1, *H. tri.* 2, *H. tri.* 3, *H. tri.* 4, and *H. tri.* 5 for *Hypericum triquetrifolium* from Bou Arada, Bahra, Dhrea Ben Jouder, Fernana and Fondouk Djedid, respectively.

Aerial parts (the top 25 cm) of the plant have been collected during the full blooming stage from five different Tunisian localities between June and July, 2008. In brief, plant samples were air-dried at room temperature and kept in darkness for one week. Then, samples (500 g) were cut in small pieces and subjected to hydrodistillation for 3h, using a standard apparatus recommended in the European Pharmacopoeia. The obtained oils were stored at +4°C in glass vials until analysis. The resulted oils were studied for their chemical variability using Gas Chromatography – Electron Ionization Mass Spectrometry (GC-EIMS) and GC coupled with Electron Ionization Mass Spectrometry (GC/EIMS). The results were reported in our previous work [38].

**Cells and tested microorganisms**

**Cell line.** The Vero cell line was derived from the kidney of a normal, adult, African green monkey (Cercopithecus) in 1962, by Yasumura and Kawakita at the Chiba University in Japan. This cell line has been used extensively for virus replication studies and plaque assays. Vero cells (kindly provided by Pr. Bruno Pozzetto, Laboratory of Bacteriology-Virology, Saint-Etienne, France) were used for culturing enterovirus strains. Vero cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), L-Glutamin (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were incubated at 37°C in a 5% CO₂ humidified atmosphere.
Bacterial and fungal strains. Gram (+) and Gram (–) bacterial strains were used in the present study (Table 1). In addition, fungal and yeast strains and isolates were included for the analysis of fungicidal activity of the obtained essential oils (Table 2).

Virus strain. Coxsakievirus B3 Nancy strain (kindly provided by Pr. Bruno Pozzetto, Laboratory of Bacteriology-Virology, Saint-Etienne, France) was propagated in Vero cells. In bref, 100 µL of the virus suspension was used to infect a confluent monolayer of Vero cells in 75 cm² culture flask and adsorbed for 1h to allow the viruses to adhere onto the cells. Non-adherent particles were washed off using 2% RPMI 1640 medium and the infected cells overlaid with 20 mL of 2% RPMI 1640 and incubated again until full cytopathic effect was observed in 5 to 6 days. The harvested virus was stored at –70°C until used.

Antimicrobial activities

Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC). The minimum inhibitory concentration (MIC) values for each essential oil on each bacterial strain and environmental isolate were determined, according to the standard protocols [35]. The bacterial strains were cultivated in tryptic soy broth (TSB) or agar (Sigma, Tunis, Tunisia) at the appropriated temperature of the strain (30°C or 37°C). Inocula were prepared by adjusting the turbidity of each bacterial culture to reach an optical density similar to a 0.5 McFarland standard, corresponding to approximately 1 – 5 x 10⁸ CFU/mL. The concentration of spore suspensions was determined by using a haematocytometer (Thoma cell) and adjusted to 1 – 5 x 10⁷ spores/mL. The broth dilution method was carried out in 96-well microtitre plates, using microbial reference
strains and field isolates. The essential oils were aseptically prepared and transferred to sterile 96-well microtitre plates by two-fold serial dilutions using 5% dimethylsulfoxide (DMSO) and then diluted in TSB. The resulted concentrations of the tested essential oils ranged between 2 and 250 µg/mL. Eighty microliters of the prepared oil suspension were added to each well, followed by 10 µL of each oil concentration and 10 µL of resazurin indicator solution (7-Hydroxy-3H-phenoxazin-3-one 10-oxide), which allows the detection of microbial growth in extremely small volumes of solution in microtitre plates without the use of a spectrophotometer. Two control wells were used for each plate: one well contained microorganism and resazurin; the second well contained only medium and resazurin in order to check the sterile conditions of the experiment. The plates were incubated anaerobically at 37°C for 24 h. All the experiments were carried out in triplicate. After incubation, bacterial growth was evaluated by color change from blue to pink. The lowest concentration indicating inhibition of growth was recorded as the MIC.

To determine the MBC, 10 µL of each culture medium with no visible growth were removed from each well and inoculated in TSB plates. The CFU number of surviving organisms was determined after aerobic incubation at the appropriated temperature during 16 – 20 h [39].

**Minimum Inhibitory and Minimum Fungicidal Concentrations (MIC and MFC).** The fungicidal activity was performed as discussed above. The exception was that the fungi and the yeast strains were cultured on malt extract broth (MEB) or agar (Fluka, Madrid, Spain) and incubated at 28°C. The essential oils (diluted in 5% DMSO) at different
concentrations were mixed with MEB and the plates were incubated anaerobically at 25°C for 48 h.

Cytotoxicity assay. The evaluation the cytotoxic effect of the essential oils is based on the reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), by the mitochondrial dehydrogenase of viable cells, to give a blue formazan product which can be measured spectrophotometrically [40]. The MTT colorimetric assay was performed in 96-well plates. Cells were seeded in 96-well plates at a concentration of 5 x 10^4 cells per well and incubated for 24 h at 37°C in a 5% CO₂ humidified atmosphere. After treatment with various concentrations of the essential oils (0.19, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, and 25 mg/mL), the cells were incubated for an additional 48 h at 37°C. The cells were examined daily under a phase-contrast microscope to determine the minimum concentration of compound that induced alterations in cell morphology. At this stage, the medium was removed and cells in each well were incubated with 100 µL of MTT solution (5 mg/mL) for 3-4 h at 37°C. MTT solution was then discarded and 50 µL DMSO were added to dissolve insoluble formazan crystal. Optical density (OD) was measured at 540 nm using a standard microplate reader (BIO-TEK® ELx800™ Universal Microplate Reader, NY, USA). Cell viability was expressed with respect to the absorbance of the control wells (untreated cells), which were considered as 100% of absorbance. The percentage of cytotoxicity is calculated as \([[(A-B)/A] \times 100\); where A and B are the OD_{540} of untreated and treated cells, respectively. The 50% cytotoxic concentration (CC_{50}) was defined as the compound's concentration (µg/mL) required for the reduction of cell viability by 50%, which were calculated by regression analysis.
**Virus inhibition assay.** In this assay, essential oils were tested to see if they either cure infected cells or protect them from infection. The experiment is simple, and relies on a cell culture system able to support virus growth. Confluent Vero cell cultures were treated with the essential oils at three different concentrations (CC$_{50}$, ½ CC$_{50}$, ¼ CC$_{50}$) during and after virus infection in two sets of experiments as follows: (Experiment 1) 5 x $10^4$ TCID$_{50}$ of the virus was exposed to three concentrations (CC$_{50}$, 1/2 CC$_{50}$, 1/4 CC$_{50}$) of each essential oil for one hour at 37°C. Then 100 μL of the mixture were added to the cells cultured fluently in 96-well flat-bottom microtiter plate; (Experiment 2) Cells were treated with three concentrations (CC$_{50}$, 1/2 CC$_{50}$, 1/4 CC$_{50}$) of each essential oil (100 μL) for one hour at 37°C. Then 5 x $10^4$ TCID$_{50}$ of the virus (100 μL) were added. Alltogether, the experiment aims to test the mode of action of the tested essential oils and to evaluate any effect of the essential oils on the virus (Experiment 1) or on the cells before infection (Experiment 2).

All plates were incubated at CO$_2$-incubator for 48 hrs. The viability of the infected and non-infected cells was evaluated using MTT reduction assay, as discussed above. The percent of protection was calculated as follows:

$$\text{Percent protection} = \frac{[(\text{ODT}) \text{ V} - (\text{ODC}) \text{ V}] / [(\text{ODC}) \text{ M} - (\text{ODC}) \text{ V}] \times 100}{\text{Where} \ (\text{ODT}) \text{ V, (ODC) V and (ODC) M indicate absorbance of the sample, the virus-infected control (no compound) and mock-infected control (no virus and no compound), respectively [41].}}$$

**Results**
**Antibacterial activity**

As shown in Table 3, the tested essential oils exhibited different antimicrobial activity with respect to geographical regions.

The essential oil of *H. triquetrifolium* from Fondouk DJe (F. DJ.) showed a potent antibacterial activity against the tested strains (MIC’s range: 0.39 – 1.56 mg/mL; MBC’s range: 1.56 – 6.25 mg/mL) except for *Vibrio cholerae* (MIC = MBC = 25 mg/mL).

The essential oil of *H. triquetrifolium* collected from Bou Arada (B. A.) was comparatively more bacteriostatic against Gram (–) bacterial strains (MIC’s range: 0.39 – 12.5 mg/mL; MBC’s range: 6.25 – 25 mg/mL). However, the highest bactericidal effect was detected against *S. aureus* (MIC = MBC = 3.12 mg/mL).

The essential oils obtained from western region, Bahra (Bah.) and Fernana (Fer.), were active to a lesser extent. Essential oils from Bahra showed good activity against *E. feacalis* and *A. hydrophila* (MIC = 0.39 mg/mL for both strains; MBC’s range: 0.39 – 3.125 mg/mL, respectively).

The essential oils from Dhrea Ben Jouder (D.B. J.) exhibited weaker antimicrobial activity against the tested strains (MIC’s range: 12.5 – 25 mg/mL; MBC’s range: 12.5 – 25 mg/mL), except for *Vibrio cholerae*, against which a significant activity was detected (MIC = 0.781 mg/mL; MBC = 3.125 mg/mL).

All together, excepting the essential oils from F.DJ., all the remaining tested samples showed a bacteriostatic effect against *V. cholerae* with MIC’s values ranged between 0.39 and 12.5 mg/mL. MBC’s values were 3.125 mg/mL and 25 mg/mL for the essential oils from Fer./D.B.J., and B.A./Bah., respectively. In addition, *B. cereus, S. epidermidis, E. coli*, and *V. alginoliticus* were resistant for all the tested essential oils, but sensitive to
the essential oils from F.DJ., with MIC’s and MBC’s values ranged between 6.25 and 25 mg/mL.

Antifungal activity

As shown in table 4, the H. triquetrifolium essential oils exhibited better antifungal and candidal activities than antibacterial activity with MIC’s values ranged between 0.39 and 12.5 µg/mL whereas MFC’s values ranged between 1.56 and 25 µg/mL. The essentials oil from B.A. showed the best fungistatic activity with MIC’s values of 0.39 µg/mL and 3.125 µg/mL for candidal and filamentous strains (Aspergillus niger, Fusarium solani, and Botrytis cinerea), respectively. The best antifungal activity was shown against C. glabrata (MFC = 1.56 µg/mL).

The Essential oils from the Estern region of Tunisia (F.DJ. and D.B.J.) showed a potent antifungal activity against the tested candidal strains with MIC’s values ranging between 1.56 and 6.25 µg/mL. The essential oils from F.DJ. showed better antifungal activity against filamentous fungal strains (MIC = 3.125 µg/mL, MFC = 3.125 µg/mL) than the essential oils from D.B.J. (MIC = 6.25 µg/mL, MFC = 6.25 µg/mL). In addition, the essential oils from F.DJ. (MIC’s range: 1.56 – 6.25 µg/mL, MFC = 6.25 µg/mL) showed slightly higher anticandidal activity than the essential oils from D.B.J. (MIC’s range: 1.56 – 3.125 µg/mL, MFC = 25 µg/mL).

Compared to the eastern regions, essential oils from the western localities (Bah. and Fer.) were less fungistatic with MIC’s values ranging between 3.125 and 12.5 µg/mL, but they have a similar fungicidal activity, with MFC’s values ranging between 3.125 and 12.5 µg/mL.
Cytotoxicity test and antiviral activity

The cytotoxic effect of the tested essential oils was dose-dependent (Figure 1). The Hypericum essential oils showed different cytotoxic profiles. The most cytotoxic essential oil was from D.B.J. (CC$_{50} = 0.58$ mg/mL) followed by Fer. (CC$_{50} = 1.12$ mg/mL), Bah. (CC$_{50} = 2.5$ mg/mL), and F.DJ. (CC$_{50} = 4.17$ mg/mL). The less cytotoxic effect was shown for the essential oil from B.A. (CC$_{50} = 12$ mg/mL) (Table 5). Unfortunately, the tested essential oils in the present study did not show an evident antiviral activity against coxsakievirus B3 Nancy strain whether incubated with virus prior to infection or incubated with cells before inoculation. We can not exclude at all the antiviral activity of these essential oils against other viruses mainly the enveloped particles, which were known to be more sensitive to the environmental conditions.

Discussion

The antibacterial activity against essential oils of Hypericum species was well documented in the literature for H. calycinum [42], H. kouytchense [43], H. coris [44], H. barbatum, H. richeri (published as H. alpinum) [45], H. rumeliacum [46], H. hyssopifolium ssp. elongatum (syn: H. elongatum) [47], H. Hyssopifolium ssp. Hyssopifolium ssp. Hyssopifolium [48], H. Hyssopifolium ssp. Microcalycinum, H. Lysimachioide var. Lysimachioides [49], H. scabroides, H. triquetrifolium [50], H. maculatum [51], H. perforatum [52], H. hirsutum [53] and H. cordatum [54]. The antibacterial activity of H. triquetrifolium was reported against Bacillus brevis, Bacillus cereus, Escherichia coli PBR 322, Escherichia coli PUC 9, Pseudomonas
aeruginosa and Staphylococcus aureus [50]. However, the antimicrobial activity were generally influenced by the type of the assay used [55]. The major components of the essential oils are found to reflect quite well their biophysical and biological features [56]. Among the pure compounds detected in these essential oils, antimicrobial activities of pure compounds of α-pinene, camphene, β-pinene, myrcene, β-cymene, limonene, γ-terpinene, borneol, 1-terpinen-4-ol, α-terpineol, geraniol, caryophyllene oxide, longiborneol, and sclareol have been well-documented [27, 57-63]. The yield percent of compounds discussed above were 26.7, 22.8, 19.6, 19.5, and 18.5 in the H. triquetrifolium essential oil from B.A., F.DJ., Fer., Bah., and D.B.J., respectively [38]. This yield may explain the good antimicrobial activities of the essential oils from B.A. and F.DJ.

The antimicrobial activity of the studied essential oils may be attributed to their major compounds however; we cannot exclude the synergistic and antagonistic effects among the chemical compounds which may lead to the completely or partially inhibition of one compound in the favor of another.

The antifungal and anticandidal activities were shown to be higher than the antibacterial activity for all the tested essential oils. The essentials oil from B.A. showed good fungistatic activity against candidal strains with MICs values ranging between 0.39 µg/mL and 3.125 µg/mL, followed by essential oils from F.DJ. and D.B.J. with MICs values ranging between 1.56 and 6.25 µg/mL whereas essential oils from Bah. and Fer. were less fungistatic with MIC’s values ranging between 3.125 and 12.5 µg/mL. The best fungicidal effect of the essential oil from B.A. was shown against C. glabrata (MFC = 1.56 µg/mL). The essential oil from F.DJ. showed better fungicidal activity against filamentous strains (MFC = 3.125 µg/mL) than the essential oil from D.B.J. (MFC = 6.25
µg/mL). The essential oils from the western localities (Bah. and Fer.) showed similar fungicidal activity, with MFC’s values ranging between 3.125 and 12.5 µg/mL. Similar study has reported the antifungal activity of Hypericum triquetrifolium against Candida albicans using disk diffusion assay [37].

Unfortunately, the tested essential oils of Hypericum triquetrifolium collected from Tunisia did not show any clear anti-enteroviral activity. However, their antiviral activity against other viral agents can not be excluded as reported previously for Hypericum connatum, Hypericum caprifoliatium and Hypericum polyanthemum against lentiviruses [64].

**Conclusion**

Antibiotic-resistant bacteria and fungi continue to be of major health concern world-wide. Bacteria have progressively developed resistance. Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic and antifongic therapy.

As far as we know, the present work is the first study reporting the antimicrobial activity of Hypericum triquetrifolium in Tunisia.

The essential oils of Hypericum triquetrifolium collected from different Tunisian localities showed promising activity against bacterial and fungal strains at non cytotoxic concentrations and merit worth consideration in future evaluation of Tunisian natural products for their antimicrobial potential. However, we were not able in this study to determine the mechanism(s) underlying behind these activities.
Unfortunately, these essential oils did not show any antiviral activity against coxsakievirus B3 Nancy strain, known to be resistant in the environment. However, the tested essential oils may exhibit antiviral activities against other viral strains mainly the enveloped viruses such as herpes virus.

**Competing interests**

The authors declare that there are no conflicts of interest.

**Authors’ contributions**

RZ carried out the studies, acquired the data, performed the data analysis, and drafted the manuscript. KS performed the bacterial assay. YT has carried out the experimental procedures of the antifungal activities. AN performed the antiviral activity and cytotoxic test. CPL and FG helped in the analysis and interpretation of the obtained results. AM revised and supervised the work.

**Knowledgments**

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**References**


Table 1. Bacterial reference strains used in the present study and their pathological effects

<table>
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<tr>
<th>Bacterial strains</th>
<th>Catalogue number</th>
<th>Effects</th>
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<tbody>
<tr>
<td><em>Bacillus cereus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ATCC 11778</td>
<td>Foodborn</td>
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<tr>
<td><em>Escherichia coli</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ATCC 35218</td>
<td>Foodborn</td>
</tr>
<tr>
<td><em>Vibrio alginolyticus</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ATCC 17749</td>
<td>Intestinal diseases, wound and ear infections</td>
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<tr>
<td><em>Vibrio cholerae</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ATCC 39315</td>
<td>Cholera</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ATCC 27853</td>
<td>Gastrointestinal diseases</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CIP 104115</td>
<td>Typhoid fever</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ATCC 7966</td>
<td>Gastroenteritis and Cellulitis</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ATCC 29212</td>
<td>Endocardites</td>
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<tr>
<td><em>Staphylococcus aureus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ATCC 25923</td>
<td>Foodborn, scalded skin syndrome</td>
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<tr>
<td><em>Staphylococcus epidermidis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CIP 106510</td>
<td>Nosocomial</td>
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<sup>a</sup>Gram (+) bacteria

<sup>b</sup>Gram (−) bacteria
Table 2. Fungal and candidal strains used in the present study and their effects

<table>
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<th>Fungal and yeast strains</th>
<th>Catalogue number/ isolates</th>
<th>Effects</th>
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<tr>
<td><strong>Aspergillus niger</strong></td>
<td>CTM 10099</td>
<td>Black mold on certain fruits and vegetables, contaminant of food, aspergillosis, otomycosis, damage to the ear canal and tympanic membrane.</td>
</tr>
<tr>
<td><strong>Fusarium solani</strong></td>
<td>Isolated from Tomato plants</td>
<td>Damping off on certain fruits and vegetables, keratitis, endophthalmitis, cutaneous infections, burn patients, mycetoma, onychomycosis, sinusitis, pulmonary disease, endocarditis, catheter infections, and septic arthritis</td>
</tr>
<tr>
<td><strong>Botrytis cinerea</strong></td>
<td>Isolated from strawberry fruit</td>
<td>Winegrower’s lung, Hypersensitivity pneumonitis, Grey mould affects many plant species</td>
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<tr>
<td><strong>Candida albicans</strong></td>
<td>ATCC 90028</td>
<td>Candidiasis, opportunistic oral and genital infections</td>
</tr>
<tr>
<td><strong>Candida glabrata</strong></td>
<td>ATCC 90030</td>
<td>Pathogen for the urogenital tract, and for the bloodstream (fungemia)</td>
</tr>
<tr>
<td><strong>Candida krusei</strong></td>
<td>ATCC 6258</td>
<td>Fungemia, nosocomial pathogen</td>
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</table>
### Table 3. Antibacterial activity of essential oils of *H. triquetrifolium* (MIC / MBC; mg/mL)

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<tr>
<td><em>Bacillus cereus</em> ATCC 11778&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25/25</td>
<td>12.5/12.5</td>
<td>25/25</td>
<td>6.25/12.5</td>
<td>1.56/3.125</td>
</tr>
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<td><em>Enterococcus faecalis</em> ATCC 29212&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>6.25/12.5</td>
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<td>12.5/25</td>
<td>6.25/25</td>
<td>0.78/3.125</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> CIP 106510&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>25/25</td>
<td>12.5/25</td>
<td>25/25</td>
<td>1.56/3.125</td>
</tr>
<tr>
<td><em>Vibrio alginoliticus</em> ATCC 17749&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5/25</td>
<td>12.5/12.5</td>
<td>12.5/12.5</td>
<td>6.25/12.5</td>
<td>1.56/3.125</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 35218&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5/25</td>
<td>25/25</td>
<td>25/25</td>
<td>6.25/12.5</td>
<td>1.56/6.25</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em> ATCC 39315&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>12.5/25</td>
<td>0.78/3.125</td>
<td>3.125/3.125</td>
<td>25/25</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>12.5/12.5</td>
<td>25/25</td>
<td>6.25/6.25</td>
<td>0.39/1.56</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> CIP 104&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>6.25/25</td>
<td>25/25</td>
<td>6.25/25</td>
<td>0.78/6.25</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em> ATCC 7966&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.39/3.125</td>
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<td>6.25/12.5</td>
<td>3.125/6.25</td>
</tr>
</tbody>
</table>

Table 4. Antifungal activity of essential oils of *H. triquetrifolium* against fungal and yeast strains (MIC / MFC; µg/mL)

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
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<td>12.5 / 12.5</td>
<td>6.25 / 6.25</td>
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<td>3.125 / 3.125</td>
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<td><em>Candida krusei</em> ATCC 6258</td>
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<td>6.25 / 6.25</td>
<td>3.125 / 25</td>
<td>3.125 / 6.25</td>
<td>6.25 / 6.25</td>
</tr>
<tr>
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<td>6.25 / 6.25</td>
<td>3.125 / 25</td>
<td>6.25 / 6.25</td>
<td>3.125 / 6.25</td>
</tr>
<tr>
<td><em>Candida glabrata</em> ATCC 90030</td>
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<td>3.125 / 6.25</td>
<td>1.56 / 25</td>
<td>6.25 / 6.25</td>
<td>1.56 / 6.25</td>
</tr>
</tbody>
</table>

MIC: Minimum Inhibitory Concentration; MBC: Minimum Fungicidal Concentration.

Table 5. 50% cytotoxic concentrations (CC$_{50}$) of *Hypericum triquetrifolium* essential oils collected from different localities.

<table>
<thead>
<tr>
<th>Essential oils from different localities</th>
<th>CC$_{50}$ (mg/mL)</th>
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</thead>
<tbody>
<tr>
<td>H. tri. B.A.</td>
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<tr>
<td>H. tri. Bah.</td>
<td>2.5</td>
</tr>
<tr>
<td>H. tri. D.B.J.</td>
<td>0.58</td>
</tr>
<tr>
<td>H. tri. Fer.</td>
<td>1.12</td>
</tr>
<tr>
<td>H. tri. F.Dj.</td>
<td>4.17</td>
</tr>
</tbody>
</table>

Figure legends:

**Figure 1.** The % viability of cells treated with *Hypericum* essential oils at different concentrations.
Figure 1. The % viability of cells treated with *Hypericum* essential oils at different concentrations.
Additional files provided with this submission:

Additional file 1: Table 1.doc, 35K
http://www.biomedcentral.com/imedia/8272034137642735/supp1.doc
Additional file 2: Table 2.doc, 34K
http://www.biomedcentral.com/imedia/6614968837642737/supp2.doc
Additional file 3: Table 3.doc, 42K
http://www.biomedcentral.com/imedia/9820285847642747/supp3.doc
Additional file 4: Table 4.doc, 38K
http://www.biomedcentral.com/imedia/1172720376427390/supp4.doc
Additional file 5: Table 5.doc, 32K
http://www.biomedcentral.com/imedia/2080905257642748/supp5.doc