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Use of *Origanum vulgare* Essential Oil as an Antibacterial Additive in the Preservation of Minced Meat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The essential oil extracted by hydrodistillation from the aerial parts of *Origanum vulgare* L. harvested in the region of Skikda (North-East-Algeria) gave an excellent oil yield (2.8%). Its analysis by Gas Chromatography–Mass Spectrometry (GC/MS) identified 98.10% of its constituents. The major components are: carvacrol (47.6%), thymol (16.6%), *p*-cymene (13.5%) and γ-terpinene (11.2%).
The aim of this study was to the preservative effect of *Origanum vulgare* essential oil applied to a very low concentration of 0.025% (minimum inhibitory concentration (MIC)), of a sensitive food of

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essential nutritional value, of great consumption and easily perishable “minced meat”, stored at different times, namely: T_0 = 0 min, T_1 = 40 min, T_2 = 24 h, and T_3 = 48 h. By studying its microbiological quality by determining the rate of reduction of the total aerobic mesophilic microflora and of *Staphylococcus aureus*. The addition of *Origanum vulgare* essential oil to minced meat allowed a highly significant reduction of $0.01<p<0.1$ in total aerobic mesophilic microflora reduction rate and very highly significant ($p\leq0.01$) for *Staphylococcus aureus*, and markedly increase the shelf life. This allows us to propose the use of this essential oil as a source of natural preservative substances.

Keywords: Origanum vulgare; essential oil; chemical composition; minced meat; microbial contamination; preservative.

1. INTRODUCTION

Food products are often exposed to the problems of contamination by microorganisms, leading to considerable economic losses and health risks that can endanger human life. Red meat is undoubtedly one of the foods most exposed to this scourge. For nutritional reasons, red meat occupies a prominent place in human diets [1]. Its composition of water and proteins of high biological value makes it a very favorable niche for the development of microorganisms which can reach a dangerous threshold [2].

As a result, it is the subject of growing concern in modern society. In the past it was preserved by drying, salting or smoking [3]. Its high biological value makes it a favorable ground for microbial proliferation, including potentially pathogenic bacteria. The latter can be the source of infection or poisoning when they produce enterotoxins [4,5].

Much microbiological research has been carried out on meat and has made it possible to isolate, by stage of production, several types of bacteria depending on whether it is fresh meat, minced meat or meat preparations [2,6].

Preservation of contamination by refrigeration or the addition of antibacterial preservatives shows many limitations. They are a source of health problems, including toxicological and teratogenic effects [7,8]. In addition, several bacteria have acquired the capacity to multiply at 4°C, such as, *Listeria monocytogenes* [9] and *Francisella tularensis*.

To remedy this problem, several authors have resorted to natural substances of plant origin.

Oregano, a spontaneous plant, abundant in the North-East of Algeria, and part of the Algerian culinary heritage, is also an important source of bioactive molecules [10]. Among these substances, essential oils of complex chemical composition, and rich in active compounds, act on various cellular targets. This contributes to the resolution of the growing issue of bacterial resistance.

Oregano essential oil contributes to the improvement of taste qualities, and classified "Generally recognized as healthy" and approved for food use by the United States Food and Drug Administration (Food and Drug Administration) [11-13].

The application of oregano essential oil, (a plant used fresh or dry as a spice, in certain meat dishes, both for its preservation qualities and its tasty taste) in the control of microorganisms, could reduce the risk spoilage and ensure food safety for consumers.

We suggest the use of *Origanum vulgare* essential oil by research into the preservative effect (applied at a low concentration) of a perishable and high-risk commodity: "minced meat". The results of the experiments will make it possible to calculate the bacterial reduction capacity in this foodstuff and to study its activity over time.

2. MATERIALS AND METHODS

2.1 Plant Material (*Origanum vulgare* Essential Oil)

The essential oil of the aerial parts of *Origanum vulgare*, collecting from the region of Hammadi Krouma (Skikda, North-East, Algeria), was obtained by hydrodistillation using a Clevenger type device, and identified by gas chromatography–mass spectrometry GC / MS). The plant was identified by Dr. Hicham Boughendjioua at the Department of Natural Sciences, Higher School of Professors for

Technological Education, Skikda, Algeria. The voucher specimen under the plant's name was deposited in the herbarium. We gave also the following voucher specimen number: (Boughendjioua 01/PPL/ 2020).

2.2 Gas Chromatographic-mass Spectral Analysis

Chromatographic analysis of the extracted essential oil was performed on gas chromatography–mass spectrometry Shimadzu QP 2010. Column used is SE - 30 type 25 m in length, 0.25 m in diameter internally, the film thickness is 0.25 microns. The injection mode is split. Helium was used as carrier gas at a constant pressure of 25.6 KPa. The temperatures of the injector and transfer line were brought to 250°C. The oven temperature was programmed according to the following conditions:

Initial column temperature was 60°C, increasing the temperature of 3°C / min to 120°C, and is kept isothermal for 5 minutes then increased to 10°C / min to 180°C. The injected volume was 1 µL.

The analysis was performed in mode electron impact (EI) ionization with ionization energy of 70 EV using in scan mode (45-450 m/z). After obtaining the chromatogram of the essential oil, identification of chromatograms was done by querying the database NIST (National Institute of Standards and Technology). The internal normalization method was used for determining the amount of each component.

2.3 Use of Essential Oil as a Food Preservative

2.3.1 Tasting test

According to Burt [14], the values of minimum inhibitory concentrations (MICs) obtained "*in vitro*" should be assigned a correction coefficient ranging from 2 to 100, so that they have the same effect in a food matrix. The use of essential oils in food products is often limited by undesirable effects (strong odor, change in taste) that they can cause in the food. For this reason, it is necessary to determine the MIC of essential oil, which is capable of inhibiting bacterial growth without altering the organoleptic characteristics of the food, namely taste and smell.

We carried out preliminary tasting tests of the meat containing 0.025% essential oil. This concentration is the modal MIC (MICm) of essential oil tested "*in vitro*". The MICm is multiplied by one of the coefficients (2, 4 and 8), in order to choose the one that does not alter the taste or the smell.

The ground meat steaks added with [MIC x 2, MIC x 4 and MIC x 8] are cooked under the same conditions.

The determined sensory attributes are taste and smell, so the odor criterion is structured around two values: Strong odor, and unnoticed odor.

On the other hand, the criterion of taste was structured around three values: good, spicy and unpleasant.

Based on the results of tasting the steaks, which taste good and smell unnoticed, we were able to determine the MIC of essential oil added to the meat.

This is why preliminary tasting tests in this kind of work are essential. So, we found it useful to multiply the value of MIC, by a correction coefficient of 4.

2.3.2 Experimental protocol

We added 0.1% of our essential oil to four 30 g samples of fresh ground meat; then we analyzed the first sample at a time of T_0 = 0 min (directly after the addition of the oil) and keep the other three samples at 4°C, to count the bacterial load at different times: T_1 = 40 min, T_2 = 24 h, and T_3 = 48 h.

The same protocol was followed for the samples not added with essential oil (controls) and stored under the same conditions.

2.4 Enumeration of Bacteria

2.4.1 Enumeration of total aerobic mesophilic microflora

We looked for the total aerobic mesophilic microflora by the method of colorimetry in liquid medium (the most probable number), for the two samples, added essential oil and the control; during the different storage times, namely T_1 = 40 min, T_2 = 24 h, and T_3 = 48 h.

The inoculation was carried out using dilutions (10^{-1}), (10^{-2}) and (10^{-3}), we introduced 1 mL of the inoculum into tubes containing 7 mL of nutritive broth (a series of 04 tubes for each dilution). Homogenize the tubes well using a

shaker. The inoculated series of tubes were incubated at 37ºC for 24 hours.

Tubes showing cloudiness in the nutrient broth medium are considered positive. The final readings, as well as the enumeration of bacteria by the search for the most probable number are carried out according to the prescriptions of the Mac Grady table.

2.4.2 Enumeration of *Staphylococcus aureus*

Petri dishes containing the Chapman selective medium were inoculated with 0.1 mL of the dilution (10^{-1}) on the surface by a rake pipette. The inoculated dishes are then incubated at 37ºC for 24 to 48 hours.

Staphylococcus aureus are pigmented yellow, surrounded by a clear halo. Non-pathogenic staphylococci are characterized by transparent and slimy colonies. The number of *Staphylococcus aureus* is determined in CFU / g of sample.

2.4.3 Calculate the abatement rate

The reduction of bacteria was studied on the two food matrices: meat with added essential oil (treated) and that not added with essential oil (control). The reduction considered by this study is the difference between the number of bacteria sought in meat without essential oil added (Control), and the number of bacteria sought in meat with essential oil added, for the total aerobic mesophilic microflora and for the *Staphylococcus aureus* counted during the four stages (0 min, 4 min, 24 h and 48 h).

The reduction is expressed as a percentage; it is calculated according to the following formula:

Abatement = [(NA - N) / NA] x 100

NA: is the number of bacteria calculated in the meat not added.
N: is the number of bacteria calculated in the added meat.

2.5 Statistical Analysis of the Results

One-way ANOVA analysis of variance associated with Tukey's method by calculating the degree of significance. The confidence level is 95.00% and the difference between the means is considered: very highly significant if p≤0.01, highly significant if p is between 0.01 and 0.1; there is no significant difference if p≥0.5.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of *Origunam vulgare* Essential Oil

The yield of extracted essential oil is 2.8%. Constituting an important source of bioactive molecules.

Chemical analysis by gas chromatography–mass spectrometry (GC / MS) of this essential oil made it possible to identify a wide range of compounds reported in Table 1. This oil consists mainly of carvacrol (47.6%). and thymol (16.6%), accompanied by other constituents at relatively low levels: *p*-cymene (13.5%) and γ-terpinene (11.2%); totaling about 98.1% (Fig. 1 and Table 1).

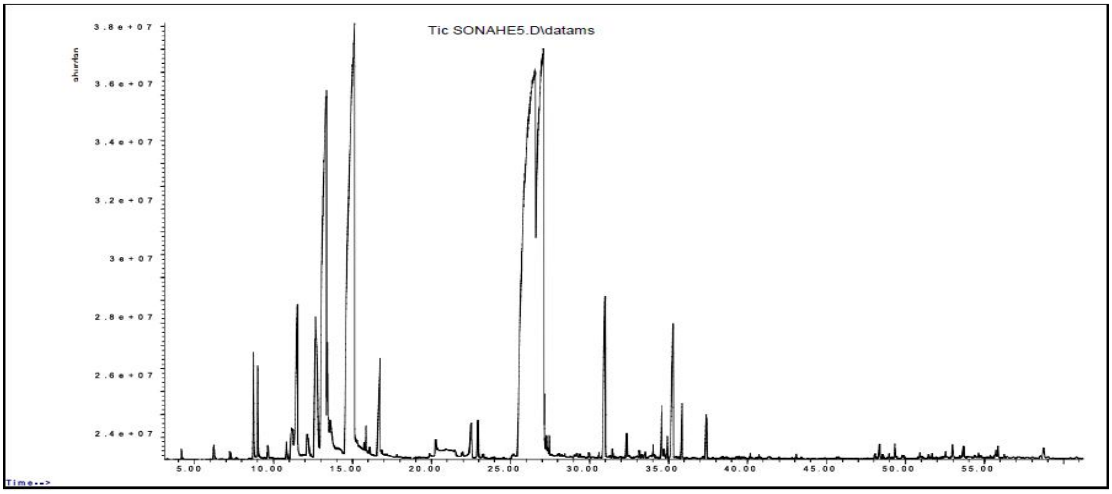

Fig. 1. Chromatographic profile of *Origanum vulgare* essential oil

Table 1. Chemical composition of *Origunam vulgare* essential oil

IK	Compounds	%	IK	Compounds	%
926	α-thujene	0.3	1027	Cis- β-Ocimene	0.1
930	α-pinene	0.7	1030	γ-terpinene	11.2
936	Camphène	0.1	1054	Terpinolène	0.1
961	Octen-3-ol	0.1	1062	Linalool	0.1
963	3-Octanone	0.2	1125	Bornéol	0.1
963	β-pinene	0.2	1132	Terpinen-4-ol	0.4
975	β-Myrcène	1.6	1145	α-Terpeneol	0.5
996	α- phellandrene	0.2	1245	Thymol	16.6
1002	α- terpinene	1.5	1275	Carvacrol	47.6
1003	*p*-cymene	13.5	1329	β-Caryophylene	1.4
1005	β-phellandrene	0.2	1492	β-Bisabolène	0.3
1009	Limonène	0.3	1502	Sesquiphellandrene	0.7
1015	3-p- Menthanone	0.6			**Total: 98.1**

The constituents of our essential oil are divided into five biochemical classes represented mainly by monoterpene phenols and monoterpene carbides (Table 2).

Table 2. Biochemical classes of *Origunam vulgare* essential oil

Biochemical classes	(%)
Phenolic derivatives	64.2
Monoterpene carbides	29.5
Monoterpene alcohols	1.1
Monoterpene ketones	0.6
Sesquiterpenes	2.7
	Total: 98.1

3.2 Enumeration of Total Aerobic Mesophilic Microflora and *Staphylococcus aureus*

We prepared two 30 g samples of fresh minced meat, one would have 0.1% essential oil added, and the other no added, would be considered a control; then we looked for the total aerobic mesophilic microflora by the colorimetry method in liquid medium (the most probable number) for the two samples, added essential oil and the control; during the different storage times, namely: T_0 = 0 min, T_1 = 40 min, T_2 = 24 h, and T_3 = 48 h. The addition of the essential oil at time T_0 reveals an immediate bacteriostatic inhibition after 40 minutes, and a lytic effect beyond 24 hours and a decrease in the number of bacteria compared to the control (Figs. 2 and 3).

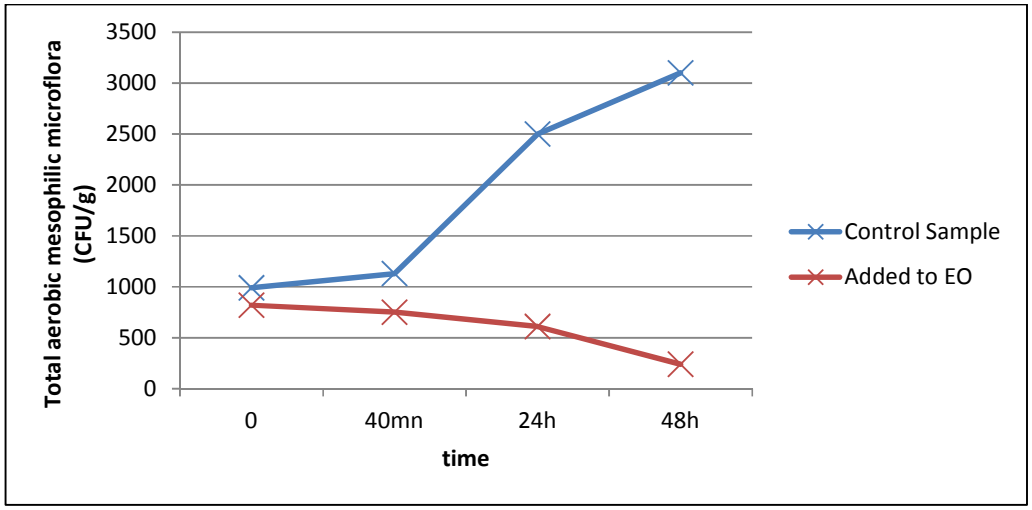

Fig. 2. Dynamics of variation of the bacterial load of minced meat at different storage times

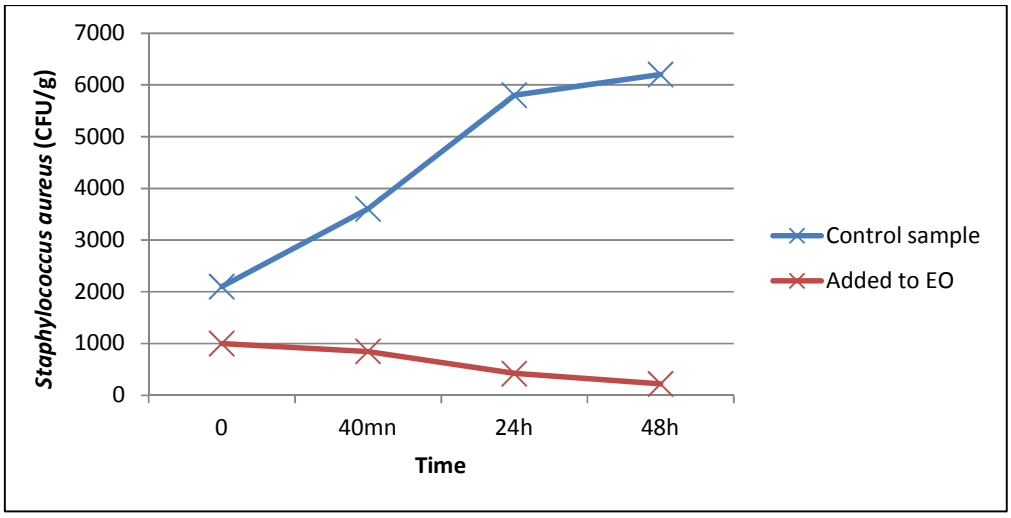

Fig. 3. Dynamics of variation of the bacterial load (*Staphylococcus aureus*) of minced meat at different storage times

At T_0, a bacterial load of total aerobic mesophilic microflora was counted, equivalent to 4.8 x 10^2 CFU / g with a presence of staphylococci of (1.2 x 10^3 CFU / g) for the sample free of essential oil (Control). *Staphylococcus aureus* are indicators of human, animal or original contamination.

The addition of essential oil to one of the samples allowed a slight reduction in the number of total aerobic mesophilic microflora (2.8 x 10^2 CFU / g); and quite large for *Staphylococcus aureus* (5.4 x10^2 CFU / g). From the results shown, it can be deduced that essential oil immediately exerts its inhibitory effect.

After 40 min of storage at 4°C, the sample not supplemented with essential oil, shows a higher bacterial load of total aerobic mesophilic microflora (9.2 x 10^2 CFU / g) and a presence of *Staphylococcus aureus* with a load of (20 x 10^3 UFC / g). Concerning the sample supplemented with essential oil, there is a decrease in the number of bacteria for total aerobic mesophilic microflora (5.1 x 10^2 CFU / g), with a remarkable decrease in staphylococci (45 x 10^2 CFU / g).

In fact, the inhibitory effect of essential oil persists, and the bacterial load of total aerobic mesophilic microflora decreases; which once again shows the antimicrobial power of the tested extract. This effect is maintained at an acceptable level despite storage time and temperature.

After 24 hours, the inhibitory effect of essential oil is maintained as well at 4°C and the number of bacteria becomes important for the sample lacking essential oil and reaches a value of (5 x10^3 CFU / g) for total aerobic mesophilic microflora. Concerning staphylococci, the bacterial load is (6.3 x 10^4 CFU / g). The addition of the additive to the sample allowed a marked reduction in the bacterial load with (25 x 10^2 CFU / g) for total aerobic mesophilic microflora and significant for *Staphylococcus aureus* (4.8 x10^3 CFU / g).

Beyond 48 hours of storage at 4°C, the bacterial load is greater, for the sample not supplemented with essential oil, it is (19 x 10^4 CFU / g) for total aerobic mesophilic microflora and reaches its maximum for *Staphylococcus aureus* (2.5 x 10^5 CFU / g). For the sample enriched with essential oil and stored under the same conditions, the reduction in the bacterial load is highly significant for total aerobic mesophilic microflora and *Staphylococcus aureus* (7 x 10^4 CFU / g), (17 x 10^4 CFU / g) respectively by compared to the control witness, without additive.

It is evident from our results that the application of *Origanum vulgare* essential oil at a low concentration which did not alter the organoleptic characteristics of the food matrix (according to the tasting test), allowed a reduction of the bacterial load for the different storage times, by addition to the control sample. It turns out that the inhibitory effect of this bioactive extract is instantaneous, it progresses over time and is

kept up to 48 hours of storage at 4°C, allowing a significant reduction (0.1<p<0.5) bacterial load of total aerobic mesophilic microflora and very highly significant (p≤0.01) of that of *Staphylococcus aureus*, according to statistical analysis (ANOVA test). From this, in the presence of essential oil and from 40 minutes up to 48 hours of storage, the number of *Staphylococcus aureus* reached an almost zero value.

This explains why Oregano essential oil exerts a very strong antistaphylococcal activity once it comes into contact with the target bacteria (bactericidal effect). This result confirms those obtained "*In vitro*".

Our study suggests the use of this type of bioactive molecules in foods as a substitute for the usual chemical preservatives; this opens the prospect of its use for the prevention and fight against the deterioration of food products stored at room temperature or at 4°C. In addition to its economic impact, this oil would contribute to the fight against food poisoning, and against multi-resistant bacteria.

The progress of the essential oil inhibitory effect could be due to the storage conditions (4°C). However, the availability of nutrients in meat such as fat, protein; antioxidant substances, salt and other substances, as well as the pH, temperature, type of packaging and characteristics of the microorganism, can undoubtedly influence the activity of essential oil. Thus, according to Holley and Holley [15], at low pH, the hydrophobicity of certain essential oils increases, which allows them to easily dissolve in the lipid phase of the bacterial membrane.

Burt [14], suggested that low water content in foods may interfere with the action of antimicrobial agents towards target sites in the bacterial cell. Thus, the high level of water and salt would facilitate the action of essential oils in meat products. By forming a protective layer of fat around bacteria or the lipid fraction in the food can absorb the antimicrobial agent by decreasing its concentration and effectiveness in the aqueous phase.

3.4 Calculation of the Reduction Rate

The reduction considered by this study is the difference between the bacterial load of total aerobic mesophilic microflora and *Staphylococcus aureus* in the sample without essential oil and that enriched with essential oil, from the results obtained, it appears that the essential oil has showed abatement rates ranging from [41% to 64%] for total aerobic mesophilic microflora and much greater for *Staphylococcus aureus* [55% to 94%] (Table 3).

Table 3. Reduction of total aerobic mesophilic microflora and *Staphylococcus aureus*

Time	Reduction of total aerobic mesophilic microflora	Reduction of *Staphylococcus aureus*
0 min	17,17 %	52,38 %
40 min	33,62 %	76,38 %
24 h	75,60 %	92,75 %
48 h	92,25 %	95,18 %

Because Oregano essential oil is among the best suited for application in meat and meat products [16], and the specificity of its antibacterial activity. This study affirms the renewed interest in essential oils which successfully demonstrates their potential use to reduce or control pathogenic flora in food products as an alternative to chemical additives.

Note that our bioactive extract rich in phenolic derivatives is very active; situation reported by Burt in his comparative study according to the contents of oils [14]. This activity is attributed to carvacrol and thymol [17-19]. Thymol being their major component binds to membrane proteins and increases permeability, destabilizing cell integrity. It also interferes with the synthesis of structural constituents [20] and energy metabolism leading to cell death [21-23]. The carvacrol thus present accentuates this effect by inhibiting the activity of ATPase [24]. Without neglecting the activity linked to monoterpene hydrocarbons, in particular *p*-cymene, and γ-terpinene which are present in sufficient quantity in our oil. They are precursors of the biosynthesis of carvacrol. They facilitate its intracellular penetration thus potentiating its action [25]. It shows a strong affinity for cytoplasmic membranes and can disrupt and affect them by causing their swelling to a greater extent than carvacrol [14]. The antibacterial activity of essential oils can also be attributed to the phenomenon of synergy between all the volatile constituents; the synergistic interactions between the different compounds can be at the origin of a much more pronounced activity than that foreseeable for the majority compounds [26].

Antibacterial activity depends on the chemical composition of essential oils and is linked to physiological bacterial groups.

4. CONCLUSION

In terms of taste, the essential oil of *Origanum vulgare* is very popular; its addition to "fresh minced meat" revealed an immediate bacteriostatic inhibition and a lytic effect beyond 40 minutes. A highly significant reduction rate of the total aerobic mesophilic microflora and very highly significant of *Staphylococcus aureus* were thus recorded. This inhibitory effect progresses over time and is maintained for up to 48 hours of storage at 4°C. The reduction is such that it is almost total for staphylococci after 48 hours. This explains why the essential oil of Oregano exerts a bactericidal effect on *Staphylococcus aureus*, confirming the results obtained "*In vitro*".

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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