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Assessment of the effects of different extraction methods on the phytochemicals, antimicrobial and anticancer activities of *Eruca sativa* extracts

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Abstract

The present study aimed to investigate the benefits of ultrasonic method of extraction compared to maceration method, on intensifying the phytochemicals, antimicrobial, and cytotoxicity activities of *Eruca sativa* leaves and sprouts ethanolic extracts. The ultrasonic treatments of *E. sativa* leaves and sprout, were tested after 10, 20 and 30 min., whereas, maceration treatments of *E. sativa* leaves and sprout, were considered after 72 h. Results of Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that *E. sativa* leaves and sprouts are reach sources of active components such as phenols and flavonoids. Moreover, the sprout macerated extracts showed higher total antioxidant activity (TAA), total phenolic contents (TPC) and total flavonoids (TFC), compared to those of the sprout ultrasonic extracts. On the contrary, the ultrasonic extraction of leaves was more efficient than maceration. Results of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity showed that ultrasonic treated leaves, sprouts (10 min.), and macerated sprouts (72 h), recorded the lowest effective concentrations (EC_{50}) of; 2.77, 3.846, and 3.676 mg/100 ml, respectively. The antimicrobial activity of the extracts was assessed using the well diffusion method. Results showed that ultrasonic treated sprouts (10 min.) and ultrasonic treated leaves (10 and 30 min.) have better antifungal activity against *Aspergillus fumigatus* (recording diameter of inhibition zone of 13 mm) and *Candida albicans* (recording diameters of inhibition zones of 13 and 11 mm, respectively). For bacteria, the *Staphylococcus aureus* was effectively inhibited (recording inhibition zone diameter of 13 mm) by the ultrasonic treated leaves (20 min.), *Bacillus cereus* was effectively inhibited with ultrasonic treated sprouts (20 and 30 min., both recording 13 mm), ultrasonic treated leaves (10 and 20 min., recording 14, 13 mm, respectively), and the macerated leaves (72 h, recording 13 mm). *Salmonella typhimurium* recorded (13 mm) with ultrasonic treated leaves (10 min.), and (12 mm) with macerated sprouts. Results of the cytotoxic potency demonstrated high activity of the macerated sprouts on HepG2 cell lines, whereas the macerated sprouts and ultrasonic treated leaves (30 min.) presented high efficacy on the HT-29 cells. Thus we concluded that extracts of *E. sativa* leaves and sprouts produced using different extraction methods, have a substantial beneficial antioxidant, antimicrobial and anticancer activities.

Keywords: *Eruca sativa*, Ultrasonic and maceration methods, Antioxidants, Antimicrobial, Anticancer

1. Introduction

Eruca sativa (*E. sativa*) (mill) is an annual edible plant known locally as "Al Jarjeer", commonly known as Rocket salad. Abdul-jalil, (2016) reported that it is a herbaceous plant of the family *Brassicaceae* (*Crucifereae*), and is greatly used as a vegetable and spice. *E. sativa* is grown all over the world, and is widely used due to its pungent flavor as an ingredient in green salads. Moreover, this plant is historically used in folk medicine as; an astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, stimulant and as an antimicrobial agent. Taviano *et al*., (2017) reported that *E. sativa* represents a good source of vitamins such as vitamin C, carotenoids, and polyphenols, which play a very important role as the natural antioxidants.

Rocket seeds are used to produce oil and have appreciated pungent taste of sprouts. A previous study conducted by Barillari *et al*., (2005) highlighted that consumption of rocket or rocket sprouts was effective in reducing oxidative stress and cardiovascular problems. Moreover, Rizwana *et al*., (2016) demonstrated that *E. sativa* seed extracts possess good antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. It is further validated by the discovery of essential bioactive compounds with antimicrobial properties, as a promising candidate for the pharmaceutical industry.

A previous study of Koubaa *et al*., (2015) proved the efficiency of solvent extracts from rocket distillate flowers (SERF) as an antibacterial agent against both Gram (+) bacteria (i.e. *B. subtilis*, *Staph. aureus*, *Enterococcus faecalis*, *B. thuringiensis*, *Micrococcus luteus*, and Gram (-) bacteria (i.e. *K. pneumonia*, *E. coli*, *S. typhimurium*, and *P. aeruginosa*). The potent ability of this plant extracts to inhibit the growth of the pathogenic bacterial strains was shown to be much better than that of the penicillin antibiotic.

In order to increase the productivity and quality of the active components extracted from plants, several intensification techniques were recently studied including; ultrasound-assisted extraction, supercritical fluids and microwave assisted extraction. Ultrasonic treatment is considered to be advantageous, due to its reduced processing time, lower energy consumption and for being eco-friendly. Wang and Weller, (2006) reported that ultrasonic treatment is simple, reliable, inexpensive and can be an effective alternative to the conventional extraction techniques. Moreover, maceration is a widely adopted method used in wine making, and is used commonly in researches about the medicinal plants. On the other hand, Nn, (2015) reported that maceration requires soaking of the plant materials (coarse or powdered) in a stoppered container with an extracting solvent, and allowing it to stand at room temperature for a period of at least 3 days with regular agitation.

The objectives of the current study were to define the possible benefits of using ultrasound treatment as a novel extraction method at different times, and to provide extracts of *E. sativa* leaves and sprouts that are more effective as antimicrobial, antioxidant and anticancer agents. The *E. sativa* ethanolic extracts obtained by the ultrasonic method were compared to those obtained with the classical maceration procedure for 72 h, concerning the extract yield, biochemical content, antimicrobial; anticancer and antioxidant activities.

2. Materials and methods

2.1. Chemicals

All chemicals and solvents used were purchased from Sigma–Aldrich, USA, Ltd.

2.2. Plant materials

About 500 g fresh leaves of *E. sativa* (Mill) were purchased from a vegetables local market, Giza, Egypt, whereas *E. sativa* seeds were purchased from Crop Research Institute, Agricultural Research Center, Giza, Egypt. These fresh leaves were washed, dried in an air oven at 50°C overnight, and then grounded in an electrical grinder. The dried leaves were stored in a plastic bag at 5°C until used.

2.3. Test microorganisms

Five bacterial strains were tested including Gram-positive bacteria; *Staph. aureus* (RCMB010010), *Bacillus cereus* RCMB 027, and Gram-negative bacteria; *Salmonella typhimurium* RCMB 006(1) ATCC 14028, *Escherichia coli* (RCMB 010052) ATCC 25955 and *Pseudomonas aeruginosa* ATCC 27853. One fungal strain (*Aspergillus fumigatus* (RCMB 002008)) and one yeast strain (*Candida albicans* RCMB 005003(1) ATCC 10231) have been used. These strains were provided by The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

2.3. Culture cell lines

Hepatocellular carcinoma cell line (HepG2) and colorectal cancer cell line (HT-29) were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). The HepG2 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM), whereas the HT-29 cells were maintained in Roswell Park Memorial Institute (RPMI) medium, supplemented with 100 mg/ml of streptomycin, 100 units/ ml of penicillin and 10 % of heat-inactivated fetal bovine serum (BSA) in humidified 5 % (v/v) CO_2 atmosphere, at 37°C.

2.4. Preparation of *E. sativa* seeds sprout

Eruca sativa seeds were cleaned from any strange materials, washed with dist. water, placed on a muslin cloth, and then continuous watering was done for a week to allow germination. According to Ali *et al*., (2018), the sprouted seeds were dried at 50°C for 48 h, ground and then stored at 5°C in plastic bags until use.

2.5. Extraction of *E. sativa* leaves and sprouts

The dried leaves and sprouts extracts were prepared according to the following procedures with some modifications:

2.5.1. Maceration extraction method

Approximately 10 g of ground *E. sativa* leaves or sprouts were soaked in 100 ml ethanol (70 %) for 24 h at room temperature. After that, the resulting extracts were filtered through filter paper (Whatman no.1). The supernatants were stored in a brown bottle, whereas the residues from the filtration were extracted again twice using the same procedure. The obtained filtrates were combined, and then evaporated in an oven at 40°C. Finally, they were placed in a refrigerator until use, in reference to Hada and Sharma, (2014).

2.5.2. Ultrasonic extraction method

About 10 g of powdered *E. sativa* leaves or sprouts were mixed with 100 ml of the extraction solvent (70 % ethanol) in a 250 ml Erlenmeyer flask. The flasks were closed and then placed individually in an ultrasonic bath (Ultrasonic Cleaner Digital-LUC-100 series) at room temperature for 10, 20, 30 min., respectively. The *E. sativa* extracts were filtered using Whatman no.1 filter paper, and then the supernatants were completely evaporated in an oven at 40°C, according to Gonelimali *et al*., (2018). Finally, they were placed in a refrigerator at 4°C till use.

The extraction yield was determined using the equation of Felhi *et al*., (2017):

$$\text{Yield (\%)} = (X_1 \times 100)/ X0$$

Where; X_1 refers to the weight of the extract after evaporation of solvent, while X0 refers to the dry weight of the plant powder before extraction.

2.6. Gas Chromatography -Mass Spectrometry (GC-MS) analysis of the extracts

The GC-MS analysis was carried out using a Gas chromatography (GC, Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000), equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm identification and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/ min.

2.7. Antioxidant assays

2.7.1. Determination of the total antioxidant activity (TAA)

The total antioxidant activity of *E. sativa* extracts was determined using the phosphor-molybdenum method according to the procedure described by Prieto *et al*., (1999). Each sample solution (0.1 ml, 0.5 mg/ ml) was combined with 0.3 ml of reagent solution (0.6 mol/ l sulfuric acid, 28 mmol/ l sodium phosphate and 4 mmol/ l ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min. After the mixture has cooled to room temperature, the absorbance was measured at 695 nm using a spectrophotometer (SpecorD250 plus- Analytik Jena) against a blank. The antioxidant activity was expressed as mg ascorbic acid equivalent/100 g extract (mg AAE/ 100 g extract).

2.7.2. Determination of the total phenolic contents (TPC) of the extracts

The total phenolic content of the *E. sativa* leaves or sprouts extracts were assessed using the Folin–Ciocalteu phenol reagent method (Singleton *et al*., 1999). About 100 µl sample extract (diluted 1:5 (v/v) with ethanol); 6 ml of dist. water and 500 µl of Folin-Ciocalteu reagent were added. After stability for 5 min. at room temperature, 1.5 ml of sodium carbonate (20 % w/v) was added. The extracts were mixed and allowed to stand for 30 min. at room temperature, before measuring the absorbance at 765 nm using a spectrophotometer (SpecorD250 plus- Analytik Jena) against a blank. Absorption was measured using three replicates. Quantitative analysis was carried out through a calibration curve using gallic acid as a reference standard. Data were expressed as gallic acid equivalents (GAE) in mg/ 100 g.

2.7.3. Assay for the total flavonoids (TFC) of the extracts

The total flavonoid content of the extracts was determined using the Aluminium chloride method as modified by Sarikurkcu *et al*., (2009). Briefly, 1 ml of 2 % $AlCl_3$ in ethanol was mixed with the same volume of the extracts. Absorption readings were taken at 415 nm after 10 min. against a blank sample (1 ml extract solution with 1 ml ethanol without $AlCl_3$).

2.7.4. Determination of the DPPH radical scavenging capacity

The ability of *E. sativa* extracts to scavenge free radicals were assayed with the use of a synthetic free radical compound 2,2-diphenyl-1-picrylhydrazyl (DPPH), in reference to Bersuder *et al*., (1998). Briefly, 500 µl of each sample was mixed with 500 µl ethanol and 125 µl 0.02 % DPPH (in 99.5 % ethanol). The mixture was shaken vigorously and then incubated in the dark. After 60 min, the absorbance was measured at 517 nm using a spectrophotometer.

The DPPH radical-scavenging activity was calculated as follows:

Radical-scavenging activity = [(A(blank)- A(sample) /A(blank)] × 100

Where; A (blank) and A (sample) are the absorbance's of the control (blank) and the sample, respectively. The effective concentration (EC_{50}) value was defined as the amount of the antioxidant necessary to inhibit DPPH radical formation by 50%.

2.8. The *in vitro* antimicrobial assay and determination of the minimum inhibitory concentration (MIC)

The *in vitro* antimicrobial assays were performed according to the National Committee for Clinical Laboratory Standards (NCCLS. 1993) recommendations. Screening inhibition tests were carried out through measuring the diameters of the inhibition zones, using the well diffusion method of Hindler *et al*., (1994). Suspensions of the microbial inocula were prepared from colonies grown overnight on agar plates, and then inoculated into Mueller-Hinton broth for bacteria, Malt broth for the fungus and the yeast strain. A sterile swab was immersed in the microbial suspensions (10^6 cfu/ ml) and used to inoculate the agar plates. The tested extracts were dissolved in dimethyl sulfoxide (DMSO) (50 mg/ ml). The inhibition zone was measured around each well after incubation for 24 h at 37°C for the bacteria, and for 5 d at 30°C for the fungi. The controls plates were prepared using DMSO. The antibiotic Gentamycin (4 µg/ ml) was used as a standard agent for the bacteria, whereas Ketoconazol (100 µg/ ml) was used for the fungus and the yeast strain.

2.9. The *in vitro* cytotoxicity assay

The *in vitro* cell viability was assessed by Sulforhodamin-B (SRB) assay according to Skehan *et al*., (1990). Aliquots of 100 µl of Hep-G2 or HT-29 cell suspensions (5×10^3 cells each) were put in 96-well plates and incubated in complete DMEM or RPMI media, respectively for 24 h. After incubation, the cells were treated with another aliquot of 100 µl medium containing the leaf extracts at various concentrations of ; 0.01, 0.1, 1, 10 and100 µg/ ml. After 72 h of the extract exposure at 37°C, the cells were fixed by replacing the medium with 150 µl of 10% trichloro-ethanoic acid (TCA), and then incubated at 4°C for 1 h. After that, the TCA solution was removed and the cells were washed 5 times with dist. water. Aliquots of 70 µl of Sulforhodamine B stain (SRB) solution (0.4 % w/v) were added, and then incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. About 150 µl of Tris (Hydroxymethyl) aminomethane (10 mM) was added to dissolve the protein-bound SRB stain. Finally, the absorbance was measured at 540 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany), in reference to Allam *et al*., (2018).

2.10. Statistical analysis

The statistical analysis of the recorded data was carried out according to Gomez and Gomez, (1984). Treatments means were compared using the least significant test at the 5 % level of probability, as outlined by Waller and Duncan, (1969).

3. Results and Discussion

3.1. Extraction yields

Table (1) shows the characteristics and extraction yields of the ethanol extracts obtained using ultrasound assisted extraction (at different times), and maceration extraction method for 72 h. Results expressed that the largest yield is obtained by maceration for *E. sativa* sprouts and leaves (25.69, 29.38 %); respectively, noting that a longer extraction time was needed for this extraction method (72 h), compared to the ultrasonic assisted method.

3.2. Analytical profiles of the *E. sativa* extracts

Results in of the GC-MS analysis presented in Table (2) show a comparison between the chemical components of the *E. sativa* leaves extracts, during

the three duration of the ultrasound extraction method. The major components are (-)-Verbenone (17.25, 21.98 and 29.19 %) at 10, 20 and 30 min., respectively, followed by γ-Sitosterol (15.78 %), cis-Trismethoxyresveratrol (10.94%) and Quercetin-3,7,3',4'-tetramethyl (10.44 %) after 10 min. of ultrasonic extraction. On the other hand, Quercetin-3,7,3',4'-tetramethyl (10.44 %) is the major component after 20 min. of extraction, followed by (-)-Verbenone (21.98 %) and 6-Methoxyluteolin (10.43 %). It is observed that ultrasonic extraction for 30 min. recorded more compounds (26), compared to the other durations of 10 and 20 min., recording 14 and 22 compounds, respectively.

Results of the GC-MS analysis of *E. sativa* sprouts ethanolic extract using ultrasonic method at different time are presented in Table (3). The major compounds recorded are (-)-Verbenone (32.02, 40.25 and 42.89 % at 10, 20 and 30 min.), respectively, whereas Vitamin E (36.69 %) and Erucic acid (14.77 %) known as fat soluble antioxidants are recorded after 10 min. of extraction. Quercetin-3,7,3',4'-tetramethyl ether (23.7 %) followed by Palmitoleic acid (10.07 %) are detected after 20 min. Finally, Elaidic acid (27.56 %) followed by Palmitoleic acid (10.31 %) are observed after 30 min.

Table 1: Characteristics and yields of the ethanolic extracts obtained using different extraction methods

Treatments	Extracts characteristics	Extracts yield (%)
ESU (10 min.)	Dark brownish semisolid	$16.67^{d} \pm 0.56$
ESU (20 min.)	Dark brownish semisolid	$17.42^{c} \pm 0.26$
ESU (30 min.)	Dark brownish semisolid	$16.44^{d} \pm 0.33$
ELU (10 min.)	Dark green semisolid	$13.058^{e} \pm 0.34$
ELU (20 min.)	Dark green semisolid	$12.39^{f} \pm 0.5$
ELU (30 min.)	Dark green semisolid	$11.53^{g} \pm 0.28$
ESM (72 h)	Dark green solid	$25.69^{b} \pm 0.17$
ELM (72 h)	Dark brownish solid	$29.38^{a} \pm 0.15$

Where; ESU= *E. sativa* sprouts ultrasonic treatment, ELU= *E. sativa* leaves ultrasonic treatment, ESM= *E. sativa* sprouts maceration treatment, ELM= *E. sativa* leaves maceration treatment. ±: express the standard deviation. Values in a column with different superscript letters are significantly different ($p< 0.05$).

Table 2: The main chemical component of the *E. sativa* leaves extracts using the Ultrasonic extraction method at different times, according to the GC-MS analysis

Rt	Name of the chemical component	Area percentage (%)		
		ELU (10 min.)	**ELU (20 min.)**	**ELU (30 min.)**
11.35	Pentadecylic acid	-----	4.16	4.47
12.85	Phytol acetate	2.21	1.33	1.34
13.05	Linoleic acid	----	0.86	0.81
13.155	17-Octadecenal	-----	1.53	1.25
13.55	Arachidonic acid	-----	3.46	3.8
13.67	6,3'-Dimethoxy-3-hydroxyflavone	----	7.77	8.35
13.8	cis-Trismethoxyresveratrol	10.94	0.71	2.3
14.33	Galangin	------	-----	0.61
14.58	citronellol	1.93	2.17	2.07
14.78	(-)-Verbenone	17.25	21.98	29.19
14.91	Octadecanoic acid	5.24	4.69	3.59
14.97	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	6.86	0.62	1.53
16.2	Erucic acid	4.94	0.97	1.45
16.25	Palmitoleic acid	-------	0.34	1.57
16.4	Arachidic acid	-------	0.68	0.94
17.2	2-Hexadecanol	-------	0.64	0.7
17.4	Vitexin	2.82	0.84	1.93
17.58	Phytanic acid	------	-------	13.6
17.62	Quercetin-3,7,3',4'-tetramethyl ether	10.44	28.43	2.07
18.5	cis-Vaccenic acid	5.71	0.65	1.49
19.36	Vitamin E	1.26	0.54	1.48
20.19	Stigmasterol	6.47	1.64	1.84
20.35	Behenic alcohol	-------	5.55	2.55
20.48	6-Methoxyluteolin	8.16	10.43	7.7
21.77	γ-Sitosterol	15.78	-------	1.61
22.5	Rhamnetin	------	-------	1.75

Where; ELU= *E. sativa* leaves ultrasonic treatment. Rt= Retention time. The assay was done in triplicates for each sample, and means of the obtained results are presented

Table 3: The main chemical component of the *E. sativa* sprouts extracts using the Ultrasonic extraction method at different times, according to the GC-MS analysis

RT	Name of the chemical components	Area percentage (%)		
		ESU (10 min.)	ESU (20 min.)	ESU (30 min.)
11.35	Pentadecylic acid	1.2	0.7	1.39
13.05	Linoleic acid	0.56	------	------
13.15	17-Octadecenal	0.57	-----	0.44
13.55	Arachidonic acid	0.73	0.67	0.46
13.67	6,3'-Dimethoxy-3-hydroxyflavone	5.93	5.9	7.51
13.8	cis-Trismethoxyresveratrol	0.43	0.87	0.49
14.78	(-)-Verbenone	32.02	40.25	42.89
14.91	Octadecanoic acid	1.34	1.92	2.16
16.2	Erucic acid	14.77	0.87	0.48
16.25	Palmitoleic acid	0.77	10.07	10.31
16.4	Arachidic acid	0.43	2.79	1.27
17.2	2-Hexadecanol	0.4	0.94	0.87
17.4	Vitexin	0.52	0.74	0.45
17.58	Phytanic acid	---	1.57	0.47
17.62	Quercetin-3,7,3',4'-tetramethyl ether	0.56	23.7	1.46
18.23	Elaidic acid	----	0.72	27.56
18.5	cis-Vaccenic acid	0.41	0.95	0.55
19.36	Vitamin E	36.69	1.5	----
20.19	Stigmasterol	0.42	-----	----
20.35	Behenic alcohol	0.48	1.54	0.5
20.48	6-Methoxyluteolin	0.64	------	0.73
21.77	γ-Sitosterol	0.5	2.92	----
22.5	Rhamnetin	0.61	1.38	-----

Where; ESU= *E. sativa* sprouts ultrasonic treatment. Rt= Retention time. The assay was carried out in triplicates for each sample, and means of the obtained results are presented.

The chemical constituents of *E. sativa* leaves and sprout ethanolic extracts obtained by maceration method are demonstrated in Table (4). The major components of the sprout extract are; (-)-Verbenone (34.21 %), cis-Vaccenic acid (32.81 %) and Erucic acid (10.93 %), while those in the leaves extract are; citronellol (18.6 %), Quercetin-3,7,3',4'-tetramethyl ether (18.08 %), (-)-Verbenone (15.3%), and 6-Methoxyluteolin (12.11 %). Similarly, Abdul-jalil, (2016) detected the presence of D, L-Citronellol in the ethanolic extract of the dried leaves of *E. sativa* by GC-MS. Moreover, using MC-MS, Jaafar and Jaafar, (2019) identified seven fatty acids in *E. sativa* seeds extracted by petroleum ether. These include; myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, erucic acid, and oleic acid. Previous study of Kim and Ishii, (2006), reported that *E. sativa* seeds have high oil contents,

protein glucosinolate and erucic acid contents, thus these seeds meal were used as animal feed in Asia, mainly in India and Pakistan. It is worth mentioning that there is a great diversity in the numbers and concentrations of the detected chemical components between the tested extracts related to their extraction methods, which interpret the current observed variation in the results of TAA, TPC, TFC and DPPH.

Table 4: The main chemical component of the *E. sativa* sprouts and leaves ethanolic extracts using the maceration method, according to the GC-MS analysis

RT	Name of the chemical component	Area percentage (%)	
		ESM (72 h)	ELM (72 h)
11.35	Pentadecylic acid	0.83	4.41
12.85	Phytol acetate	0.36	1.39
13.05	Linoleic acid	0.37	0.24
13.155	17-Octadecenal	-----	0.4
13.55	Arachidonic acid	1.01	1.51
13.67	6,3'-Dimethoxy-3-hydroxyflavone	7.32	5.9
13.8	cis-Trismethoxyresveratrol	0.48	0.31
14.33	Galangin	0.41	----
14.58	citronellol	0.52	18.6
14.78	(-)-Verbenone	34.26	15.3
14.91	Octadecanoic acid	1.74	0.86
14.97	4',6-Dimethoxyisoflavone-7-O-β-D glucopyranoside	0.79	-------
16.2	Erucic acid	10.93	0.94
16.25	Palmitoleic acid	0.83	0.86
16.4	Arachidic acid	0.34	1.19
17.2	2-Hexadecanol	-----	0.7
17.4	Vitexin	0.43	1.13
17.58	Phytanic acid	-------	1.76
17.62	Quercetin-3,7,3',4'-tetramethyl ether	1.25	18.08
18.5	cis-Vaccenic acid	32.81	1.42
18.66	Behenic acid	0.83	------
19.36	Vitamin E	0.51	0.56
20.19	Stigmasterol	2.62	3.69
20.35	Behenic alcohol	0.44	3.13
20.48	6-Methoxyluteolin	0.55	12.11
21.77	γ-Sitosterol	-----	5.5
22.5	Rhamnetin	0.38	----

Where; ESM= *E. sativa* sprouts maceration treatment, ELM= *E. sativa* leaves maceration treatment. Rt= Retention time. The assay was carried out in triplicates for each sample, and means of the obtained results are presented

3.3. Antioxidant properties of the *E. sativa* extracts

The total antioxidant activity (mg AAE /100 g), total phenolic content (mg GAE /100 g) and total flavonoid content (mg Quercetin/100 g) of *E. sativa* leaves extracts, recovered using ultrasonic extraction method at different times (10, 20 and 30 min.) and the maceration method are demonstrated in Table (5).

The TAA of *E. sativa* leaves extract is significantly ($p< 0.05$) higher in ELU (30 min.) (10234.5 mg AAE /100 g), when compared to the other treatments ELU (10 and 20 min.), and to the maceration method. It is noticed that ELU (30 min.) presented the highest antioxidant activity, probably related to its chemical profile, especially the relatively high percentage of the flavonoid compounds such as; 6,3'-Dimethoxy-3-hydroxyflavone, identified by the GC-mass analysis. This is consistent with Mooi *et al*., (2003) who considered methylated kaempferols flavonoids to show varying degrees of antioxidant activities. In addition, results of the GC-mass analysis of ELU (30 min.) revealed the presence of fatty acids including; Pentadecylic acid, Linoleic acid, Arachidonic acid, Octadecanoic acid, Palmitoleic acid, and Phytanic acid, which represent about 42.32 % of the fatty acids, when compared to the total compounds in this treatment. These acids have an antioxidant effect by lipid autoxidation that is initiated by a chain of lipophilic radicals, in accordance with Jaafar and Jaafar, (2019).

The total phenolic content of ELU (30 min.) showed significantly ($p< 0.05$) higher contents, compared to the maceration method (ELM). The total flavonoid content is significantly ($p< 0.05$) high in the ELU (20 min.), while there are no significant differences ($p< 0.05$) between the total flavonoid contents of the other three extracts. These results are coincident with the GC-Mass analysis, which showed high percentage of the Quercetin compound (28.43 %). In agreement with Xu *et al*., (2019), quercetin is confirmed to be a polyphenolic flavonoid compound that prevents various diseases including; osteoporosis, certain types of cancer, tumors, and lung and cardiovascular diseases.

Table 5: Total antioxidant activity, total phenolic content and total flavonoid content of *E. sativa* leaves ethanolic extracts using the different extraction methods

Treatments	Total antioxidant activity (mg AAE/ 100 g)	Total phenolic content (mg GAE/ 100 g)	Total flavonoid content (mg Quercetin/ 100 g)
ELU (10 min.)	$6625^{d} \pm 121.66$	$3581^{b} \pm 34.06$	$12861.67^{b} \pm 1126.41$
ELU (20 min.)	$8911.17^{b} \pm 130.32$	$3137.83^{d} \pm 8.46$	$15228.33^{a} \pm 1024.04$
ELU (30 min.)	$10234.5^{a} \pm 127.35$	$3707.33^{a} \pm 11.86$	$12078.33^{b} \pm 12.58$
ELM (72 h)	$8136.83^{c} \pm 112.33$	$3309.33^{c} \pm 7.75$	$12545.0^{b} \pm 662.51$

Where; ELU= *E. sativa* leaves ultrasonic treatment, ELM= *E. sativa* leaves maceration treatment. ±: express the standard deviation. Values in a column with different superscript letters are significantly different ($p< 0.05$). The assay was done in triplicates for each sample, and means of the obtained results are presented.

Results of the total antioxidant activity, total phenolic and total flavonoid contents in the sprouts extracts (Table 6), revealed that the maceration extraction method is highly ($p< 0.05$) significant, compared to the ultrasonic method at different times. These results may be attributed to the contribution of high percentage of 6,3'-Dimethoxy-3-hydroxyflavone (7.32 %) identified by GC-mass analysis, which is considered as methylated kaempferols flavonoid that have varying degrees of antioxidant activities (Mooi *et al*., 2003). On the other hand, Erucic acid (10.93 %) and cis-vaccenic acid (32.81 %) are considered as fatty acids related to the antioxidant activity, in accordance with Al-Douri and Shakya, (2019).

Table 6: Total antioxidant activity, total phenolic content and total flavonoid content of *E. sativa* sprouts ethanolic extracts using the different extraction methods

Treatments	Total antioxidant activity (mg AAE/ 100 g)	Total phenolic content (mg GAE/ 100 g)	Total flavonoid content (mg Quercetin/ 100 g)
ESU (10 min.)	$9871.42^{b} \pm 80.27$	$3978.58^{b} \pm 4.71$	$3929.17^{d} \pm 29.19$
ESU (20 min.)	$9656.58^{c} \pm 51.44$	$3929.83^{c} \pm 7.17$	$6215.0^{c} \pm 355.78$
ESU (30 min.)	$9222^{d} \pm 63.25$	$3901.5^{d} \pm 4.09$	$6998.33^{b} \pm 285.68$
ESM (72 h)	$10034.92^{a} \pm 45.17$	$4189.17^{a} \pm 12.63$	$13445.0^{a} \pm 230.65$

Where; ESU= *E. sativa* sprouts ultrasonic treatment, ESM= *E. sativa* sprouts maceration treatment. ±: express the standard deviation. Values in a column with different superscript letters are significantly different ($p<0.05$). The assay was done in triplicates for each sample, and means of the obtained results are presented

3.4. The DPPH scavenging activity

The antioxidant activity of the tested extracts was further assessed by determining their free radical scavenging activity against DPPH. Results showed that all extracts are capable of inhibiting the free radicals and the EC_{50} (concentration required to obtain a 50 % antioxidant effect) of the leaves and sprout extracts were determined, and demonstrated in Table (7). According to Chen *et al*., (2013), the DPPH radical scavenging activity is generally quantified in terms of inhibiting the percentage of the pre-formed free radicals by the antioxidants. The EC_{50} is typically an employed parameter to express the antioxidant capacity, and to compare the activity of different compounds. In the present study, the ELU (10 min.) showed higher inhibitory activity against DPPH, and recorded the lowest EC_{50} (2.77 mg/100 ml), compared to the other tested extracts. The scavenging activity of the ESM and ESU (10 min.) showed higher inhibitory activities against DPPH, and recorded the lowest EC_{50} (3.676 and 3.846 mg/ 100 ml); respectively, compared to the other studied extracts.

3.5. Antimicrobial potential of *E. sativa* leaves and sprouts ethanolic extracts

Currently, the antimicrobial efficacy of *E. sativa* ethanolic leaves extracts obtained through ultrasonic and maceration extraction methods were screened for Gram-positive bacteria including; *Staph. aureus* and *B. cereus*, Gram-negative bacteria including; *S. typhimurium*, *E. coli* and *P. aeruginosa*, mold fungi such as *A. fumigatus*, and yeast fungi such as *C. albicans*. A standard antifungal antibiotic (Ketoconazole) and a standard antibacterial antibiotic (Gentamycin) were used as positive controls, and their inhibition zones diameters are

listed in Tables (8, 9). All the concerned extracts demonstrated variable inhibitory potential against the tested microorganisms, as clear in Table (8, 9). It is observed that the extract of the maceration method has an inhibitory effect on *A. fumigatus* (recording inhibition diameter of 12 mm), in contrast to the ultrasonic method, which does not affect the tested fungus.

Previous study of Rizwana *et al*., (2016) highlighted that the family of *Brassicaceae* that include *E. sativa* contain major secondary metabolites such as; alkaloids, flavanoids, tannins and phenols, with recognized antimicrobial and antioxidant potency.

Table 7: Scavenging activity (EC_{50}) of *E. sativa* leaves and sprout extracts on DPPH radicals

Treatments	EC_{50} (DPPH) mg/ 100 ml	Treatment	EC_{50} DPPH mg/100 ml
ELU (10 min.)	2.777	ESU (10 min.)	3.846
ELU (20 min.)	4.545	ESU (20 min.)	4.098
ELU (30 min.)	3.731	ESU (30 min.)	4.046
ELM (72 h)	4.807	ESM (72 h)	3.676

Where; ELU= *E. sativa* leaves ultrasonic treatment, ELM= *E. sativa* leaves maceration treatment. ESU= *E. sativa* sprouts ultrasonic treatment, ESM= *E. sativa* sprouts maceration treatment. The assay was done in triplicates for each sample, and means of the obtained results are presented

Table 8: Antimicrobial activity of *E. sativa* leaves ethanolic extracts

Tested microorganisms	**Zone of inhibition (mm)**				
	Control antibiotic	**Ultrasonic method**			**Maceration method**
		ELU (10 min.)	**ELU (20 min.)**	**ELU (30 min.)**	**ELM (72 h)**
Mold fungi	Ketoconazole				
A. fumigatus	17	NA	NA	NA	12
Yeast fungi	Ketoconazole				
C. albicans	20	13	10	11	11
Gram + bacteria	Gentamycin				
Staph. aureus	24	NA	13	8	NA
B. cereus	25	14	13	12	13
Gram - bacteria					
S. typhimurium	17	13	10	11	9
E. coli	30	10	11	9	12
P. aeruginosa	27	NA	NA	NA	NA

Where; ELU= *E. sativa* leaves ultrasonic treatment, ELM= *E. sativa* leaves maceration treatment. The assay was carried out in triplicates, and means of the obtained results are presented

Table 9: Antimicrobial activity of the ethanolic extract of *E. sativa* sprouts

Tested microorganisms	Zone of inhibition (mm)				
	Control antibiotic	**ESU (10 min.)**	**ESU (20 min.)**	**ESU (30 min.)**	**ESM (72 h)**
Mold fungi	Ketoconazole				
A. fumigatus	17	13	12	NA	10
Yeast fungi	Ketoconazole				
C. albicans	20	NA	NA	NA	12
Gram + bacteria	Gentamycin				
Staph. aureus	24	NA	NA	NA	NA
B. cereus	25	12	13	13	11
Gram - bacteria					
S. typhimurium	17	14	12	11	12
E. coli	30	13	11	12	10
P. aeruginosa	27	NA	NA	NA	NA

Where; ESU= *E. sativa* sprouts ultrasonic treatment, ELU= *E. sativa* leaves ultrasonic treatment. The assay was carried out in triplicates, and means of the obtained results are presented

However, leaves extracts of both methods caused inhibition of *C. albicans* growth, recording inhibition zone diameters of; 13, 10, 11 mm at 10, 20 and 30 min; respectively, and 11 mm for the maceration treatment. The ultrasonic method caused potent inhibition on *Staph. aureus* growth (recording 13 mm) after 20 min. compared to the other durations, but the maceration method does not cause an effect. *B. cereus*, *P. aeruginosa* are affected by both extraction methods. This result coincides with that of Rizwana *et al*., (2016). Similarly, in a previous study, Balouiri *et al*., (2014) found that the methanolic extracts of rosemary and sage obtained by sonication had greater antibacterial activity than that obtained by the maceration method.

The antimicrobial activity of *E. sativa* ethanolic sprout extracts using both methods of extraction are illustrated in Table (9). Both extraction methods affected *A. fumigatus*, (recording inhibition diameters of; 13 and 12 mm at 10 and 20 min, respectively) for the ultrasonic treatment, and (10 mm) for the maceration method. The ultrasonic sprout extract showed no impact on *C. albicans*, in contrast to the maceration method on the same strain that recorded an inhibition diameter of (12 mm). In the current study, it is observed that *E. sativa* sprout extracts of both methods do not demonstrate any inhibitory potential on *Staph. aureus* and *P. aeruginosa*. On the other hand, *B. cereus* is affected by the maceration extract recording inhibition diameter of (11 mm), and by the ultrasonic extract at 10, 20 and 30 min. recording (12, 13, 13 mm, respectively). For *S. typhimurium*, it is observed that the diameter of the inhibition zone decreased gradually recording (14, 12 and 11 mm) with increasing the ultrasonic duration time (10, 20 and 30 min., respectively). For *E. coli*, the inhibition zone diameter induced by the ultrasonic sprout extracts recorded (13, 11 and 12 mm, at 10, 20 and 30 min., respectively), which is greater than that obtained by the maceration treatment (10 mm). Current results are in agreement with Dholvitayakhun *et al*., (2012), who reported that the aqueous and ethanol extracts of *Adenanthera pavonina* obtained by the ultrasonic assisted extraction were more successful than those obtained through the traditional processes. This may be attributed to the disturbance of the integrity of the plant cell walls induced by the ultrasonic method.

The current antimicrobial activities of the leaves and sprouts extracts may attributed to the presence of bioactive compounds such as erucic acid,

isothiocyanates and (-) vebrenon in these extracts, in agreement with the previous findings of Khoobchandani *et al*., (2010); Paraschos *et al*., (2011). Recently, Doulgeraki *et al*., (2017) study showed that there is a notable effect of *E. sativa* seeds on the antibacterial potential against *S. aureus* and *B. cereus*. In addition, Arribas *et al*., (2019) demonstrated that the presence tocopherols, oryzanol and β-sitosterol in the rice bran are mainly related to the antimicrobial efficacy of rice.

3.6. Minimum inhibitory concentration (MIC) of the *E. sativa* extracts

The minimum inhibitory concentrations (MICs) of ESU (10, 20 and 30 min.), ELU (10 and 20 min.) and ELM obtained by the well diffusion method against several microorganisms mainly; *A. fumigatus*, *C. albicans*, *Staph. aureus*, *B. cereus*, *S. typhimurium* and *E. coli* are shown in Table (10). The lower the value of the MIC, the more active will be the extract. The extract of ESU (10 and 30 min.) strongly inhibits *E. coli* and *B. cereus* with MIC of 390.6 mg/ ml. Whereas, ELU (20 min.) inhibit *Staph. aureus*, ESU (20 min.) and ELU (10 min.) inhibits *B. cereus*, and ESU (10 min.) inhibit *A. fumigatus* with MIC of 781.3 mg/ ml. It is found that ELU (10 min.) weakly inhibits *C. albicans* and *S. typhimurium* with MIC of 1562.5 mg/ ml, and also ELU (20 min.) weakly inhibits *B. cereus* with the same MIC.

Table 10: Minimum inhibitory concentrations (MIC) of the *E. sativa* extracts

Tested microorganisms	Treatment	MIC (mg/ ml)
A. fumigatus	ESU (10 min.)	781.3
C. albicans	ELU (10 min.)	1562.5
Staph. aureus	ELU (20 min.)	781.3
B. cereus	ESU (20 min.)	781.3
	ELU (10 min.)	
	ELU (20 min.)	1562.5
	ESU (30 min.)	390.6
	ELM (72 h)	
S. typhimurium	ELU (10 min.)	1562.5
E. coli	ESU (10 min.)	390.6

Where; ESU= *E. sativa* sprouts ultrasonic treatment, ELU= *E. sativa* leaves ultrasonic treatment, ELM= *E. sativa* leaves maceration treatment. The assay was carried out in triplicates, and means of the obtained results are presented

3.7. *In vitro* antitumor efficacy of the *E. sativa* extracts against the cancer cell lines

The cell viability assays are basically toxicological steps that explain a toxicant's cellular response. In addition, they also give information about cell death, survival and metabolic activity. Two extracts ELU (30 min.) and ESM have been chosen to examine their anticancer activities against HepG2 and HT-29 cell lines using the Sulforhodamin-B (SRB) assay, as they expressed higher antioxidant activities. The lower the IC_{50} values (the concentration of extract at which 50 % of the cell is inhibited), the more potent the extract is. These values were obtained from in vitro SRB cytotoxic bioassay, and are illustrated in Fig. (1, 2). The IC_{50} values for the HepG2 cell line are (ESM: 27.75 µg/ ml); [ELU (30 min.) >100 µg/ ml], whereas, the HT-29 cell line are [ESM and ELU (30 min.) >100 µg/ ml].

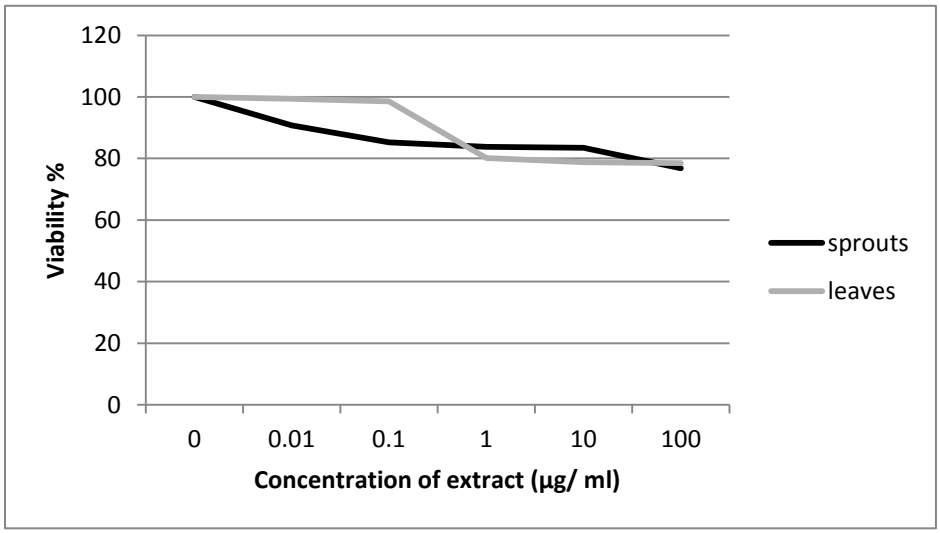

Fig. 1: IC_{50} values of ELU (30 min.) and ESM extracts against the HT-29 cell lines

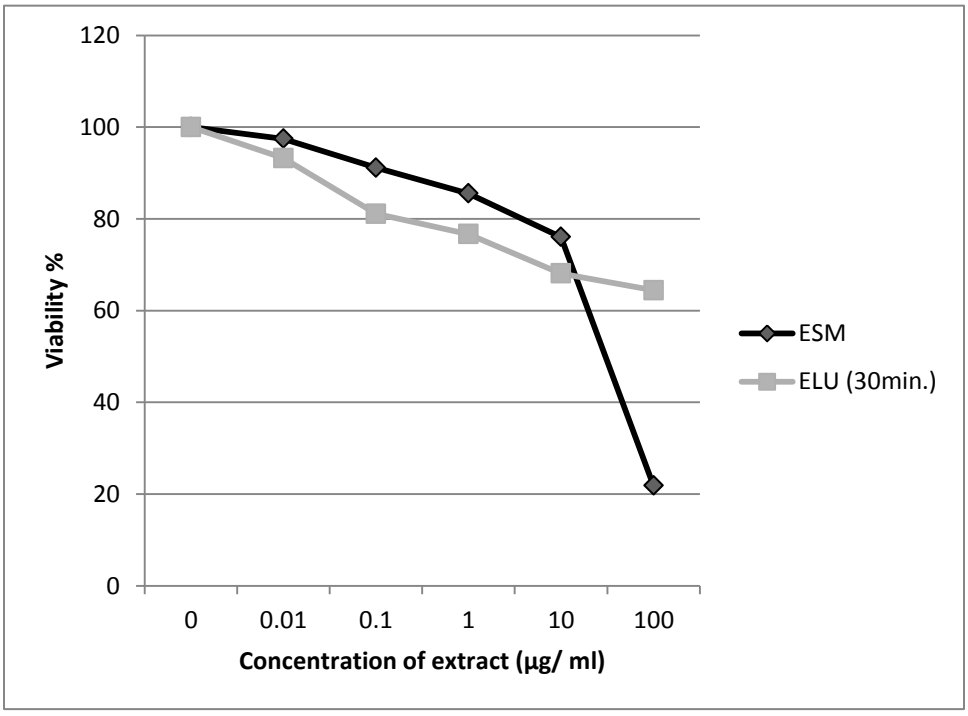

Fig. 2: IC_{50} values of ELU (30 min.) and ESM extracts against the HepG2 cell lines

Results in Table (11) demonstrated the effect of ESM and ELU (30 min.) extracts at different concentrations on the survival of HT-29 and HepG2 cell lines. The ESM at 1.0 µg/ ml, ELU (30 min.) at 10µg/ ml decreased the viability of HT-29 cells to 83 and 78 %, respectively. On the other hand, the ESM and ELU (30 min.) at 100 µg/ ml decreased the viability of the Hep G2 cells to 21 and 64 %, respectively.

Table 11: Effect of different concentrations of *E. sativa* extracts on the viability of HT-29 and HepG2 cell lines

Concentration of extracts (µg/ ml)	Survival percentage (%)			
	HT-29		HepG2	
	ESM	ELU (30 min.)	ESM	ELU (30 min.)
0	100	100	100	100
0.01	90.74	99.39	97.45	93.22
0.1	85.23	98.5	91.15	81.1
1	83.81	80.19	85.52	76.67
10	83.49	78.8	76.04	68.08
100	76.86	78.54	21.84	64.41

Where; ESM= *E. sativa* sprouts maceration treatment; ELU= *E. sativa* leaves ultrasonic treatment. The assay was carried out in triplicates, and means of the obtained results are presented

Life-threatening diseases are often cancer-related. Such diseases, including cancers are due to the reactive oxygen species, lipid peroxidation, and free radicals; however, the antioxidants that can slow or prevent this oxidation cycle and lipid peroxidation can avoid their activities. Thus, through scavenging the free radicals, the antioxidant plays a significant role in reducing the chronic diseases including; cancer and the cardiovascular diseases. Recently, Abdel-Hady *et al*., (2018) reported that plants are key sources of antioxidants, including polyphenolic compounds and saponins. Moreover, the present study revealed that ESM and ELU (30 min.) extracts contain fatty acids such as; Linoleic acid, Arachidonic acid, Octadecanoic acid, which previously reported by Youssef *et al*., (2019) to have anti-inflammatory and anticancer activities. A previous study conducted by Khoobchandani *et al*., (2011) tested the anticancer activity of the solvent extracts from the aerial, root parts and seed oil of *E. sativa* against melanoma cells. The seed oil (rich in isothiocyanates) reduced the tumor growth, compared to the control. In addition, Jin *et al*., (2009) reported that the HT-29 cell line used in this study has a higher activity of glutathione-S-transferase (GST) enzyme compared to the primary colonocytes. Therefore, the isothiocyanates produced by glucosinolate hydrolysis could be rapidly conjugated to glutathione through the action of GSTs, and therefore become non available for defense against the oxidative stress. It is thought that the anticancer potential of ESM and ELU (30 min.) extracts noted in the current study could be attributed to the presence of stigmasterol in ESM and ELU (30 min., and γ-sitosterol in ELU (30 min. These probabilities are in consistent with the recent findings of Abu-Lafi *et al*., (2019), who illustrated also that stigmasterol and γ-sitosterol are known to exhibit significant pharmacological activities,

especially anticancer, anti-inflammatory, and antibacterial ones.

Conclusion

From the present results, it can be concluded that *E. sativa* sprouts and leaves extract obtained using both ultrasound and maceration methods are abundant sources of several bioactive compounds, which can be used as sources of new and useful antioxidants, antimicrobials and anti-cancerous. The ultrasound method of extraction was more effective in extracting antioxidants, total phenol and total flavonoids from the *E. sativa* leaves, than the maceration method. However, in the case of sprouts, the maceration method was more effective to extract the antioxidants, total phenol and total flavonoids. It is observed that both methods are effective for extraction of antimicrobials from leaves and sprouts. The sprouts and leaves extracts exhibited anticancer activities against the Hepatocellular carcinoma and Colorectal cancer cell lines. These activities may be attributed to the presence of different classes of bioactive compounds such as; fatty acids, sterols, flavonoids, and phenols.

The data presented in the current study shows that *E. sativa* sprouts and leaves extracts could potentially be used in modern applications aimed at the treatment and\or prevention of foodborne diseases. Future research work is recommended to assess the application of the investigated extracts in the food preservation systems.

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Conflict of interest

The authors declare that they have no any competing interests.

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Ethical approval

Non-applicable.

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