Antioxidant, Antimutagenic and Cytotoxic Properties of Essential Oil from *Corchorus olitorius* L. Flowers and Leaf

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ABSTRACT

Background: This present research highlight the phytochemical profile, the antioxidant, the antimicrobial, the antimutagenic and the antiproliferative activities of an essential oil extracted from *Corchorus olitorius* L. flowers and leaf (COFL), an aromatic plant widely distributed in Tunisia and used as a traditional food plant. **Results:** Gas chromatography/ mass spectrometry was used to determine the composition of the COFL essential oil (COFL-EO). Forty-three components were identified and the main compounds were benzaldehyde (56%), methyl 4-methoxysalicylate (6.55%) and carvacrol (4.75%). The COFL-EO was also found to possess antioxidant activities, as evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method and β-carotene bleaching assay. The antibacterial activities showed good growth inhibition compared to positive controls. The diameter of the inhibition zones reached a maximum of 16.7 ± 0.1 mm when tested against *Salmonella typhimurium* using just 6 mg extract. On the other hand, the antimutagenic activity was assessed using the Ames *Salmonella*/microsome mutagenicity test and the COLF-EO exhibited high antimutagenic effects at 0.125, 0.0125 and 0.00125 mg/plate. Antiproliferative activity of this essential oil evaluated in four mammalian cells lines was significantly stronger in Hela cell line (IC_{50}=4 ± 0.98 µg/mL). **Conclusion**: Overall, results presented here suggest that the COFL-EO is a potential new source of active ingredients for food and pharmaceutical industry.

Keywords: Antioxidant, Antimicrobial, Antimutagenic, Chemical composition, *Corchorus olitorius* L., Cytotoxic, Essential oil.

INTRODUCTION

The emerging concepts of cancer is that the cancer cells are unstable and its unstability is brought about by the documentations of cascade of mutations caused by mutagens and suggested that mutagenesis drives out tumour progression.[1] Mutations results from the side effects of free radicals such as hydrogen peroxides, superoxide anions, and organo peroxides, etc. produced by drugs, ultraviolet radiations, ionising radiations, pollution as well as the endproducts of normal metabolic process of aerobic organisms.[2-4] The interaction of the free radicals with polyunsaturated fatty acids, nucleotides and disulphide bonds[5] has been implicated as the major factor to cause the oxidation of the biological compounds and eventually leads to mutations[6] and many degenerative diseases like emphysema, cardiovascular, inflammatory diseases, cataracts, etc.[5] Natural antimutagenic compounds obtained from edible and medicinal plants are of particular importance in this regard because they produce no undesirable xenobiotic effects on living organisms that would offset any potential usefulness in cancer prevention in humans.[7] There is a wide range of prospective human health applications for plant species that possess antimutagenic properties. Plant-based medicinal essential oils (Eos) have been the focus of many studies;

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they have been applied in food, pharmaceutical industries and are compounds of many cosmetic products. Eos are currently investigated widely because of the irrelatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use.[8] In particular, essential oil displays carminative, antiflatulence, and anticolic properties; aromatherapists use it the holistic relaxant.[9] Also, this essential oil acts as a central nervous system depressant, anticonvulsant, sedative, spasmolytic agent, local anesthetic, antioxidant, antibacterial, and mast cell degranulation inhibitor.[10-14]

Located in the North African region with Mediterranean climate, Tunisia has a breeding ground for the development of a large number of medicinal and aromatic species currently experiencing a renewed interest in their use in the pharmaceutical, food and cosmetics.[8] Thus, over than 500 species out of 2103 (approximately 25% of the total flora) are considered of therapeutic use.[15] *Corchorus olitorius* L. (*Asclepiadaceae* family) is native to the Mediterranean region and widely distributed in the Sahara area. In Tunisia, it is predominantly found in the south of the country. The young leaves of *C. olitorius* L. are edible and used as a vegetable for soup. In recent years, this plant, important as a nutritive vegetable rich in K, Ca, P, Fe, ascorbic acid, and carotene,[16] has been cultivated and consumed under the name "mloukhya" in Tunisia. It is used also as an herbal preparation because of its reputed medicinal properties, e.g. for the treatment of headaches and diabetes.[17] It has been reported to have various biological activities, such as favorable free radical scavengers[18] as well as primary antioxidants that may react with free radicals and limit reactive oxygen species attack on biological and food systems.

Therefore, the aims of this study were to determine the antioxidant and antimicrobial activities using well diffusion agar and broth microdilution methods of COFL-essential oil extracted from flowers and leaf as related to their chemical composition and to identify their antimutagenic and antiproliferative activities. To our best knowledge and literary survey, there is no report available on chemical composition analysis of essential oil derived from *C. olitorius* L (COFL), its antioxidant, antimicrobial, antimutagenic and antiproliferative properties.

MATERIALS AND METHODS

Chemicals

1,1-diphenyl- 2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), β-carotene, linoleic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), phosphate buffered saline (PBS), Dimethyl sulfoxide (DMSO), 4-nitro-o-phenylenediamine (4-NPD) and sodium azide (NaN_3) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All culture media and standard antibiotics were purchased from Bio-Rad (Bio-Rad laboratories, France). All other chemicals and solvents were of analytical grade. All solutions were freshly prepared in distilled water.

Plant material

Fresh flowers and leaf of *C. olitorius* L. were collected from Gebes (Latitude N 12 33° 53' 34" N, Longitude 10° 6' 11" E) fields (Tunisia, on Mai 20, 2013). No specific permissions were required for this location and research. This research field did not involve endangered or protected species. The voucher specimens were deposited in the *Herbarium* of the Faculty of Science and Arts, Tunis Campus University, Tunisia (FST). The raw material was washed with distilled water, dried at 80°C for 5h, grounded into fine particles and passed through 2 mm sieve screen to obtain *C. olitorius* L. leaf and flowers (COLF) powder.[19] All analyses were performed using triplicate samples and analytical results were expressed on a dry matter basis.

Extraction of the essential oil

The dried powder from COLF (100 g) was submitted to hydrodistillation for 4 h using a Clevenger-type apparatus (Staffordshire, UK). The yield was determined by weighing the remaining oil on an analytical balance. The distilled oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

GC–MS analysis

The analyses were performed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (60 m x 0.25 mm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 220°C and 290°C, respectively. The column temperature was programmed from 80°C to 220°C at a rate of 4°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gaz (Helium) was 1.0 mL/min. A sample of 1.0 μL was injected, using split mode (split ratio, 1:100). The composition was reported as a relative percentage of the total peak area. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to n-alkanes, compared to MS corresponding database (Wiley version 7.0 library) and published data and spectra of authentic compounds.

Antioxidant activity: DPPH assay

The DPPH radical-scavenging activity of COLF-EO was determined by the method of Kirby and Schmidt[20] with some modifications. A volume of 500 µL of essential oil at different concentrations (0.2–1.0 mg/mL) was added to 375 µl of 99% methanol and 125 µl of DPPH solution (0.2 mM in methanol) as free radical source. The mixture was then incubated for 60 min in the dark at room temperature. Afterwards, the absorbance measurements were read at 517 nm using a Uv-T70 spectrophotometer (T70 UV–visible spectrometer, PG instruments Ltd, Wibtoft, China). BHA was used as positive control. DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the sample), and A_{sample} is the absorbance of the essential oil. The IC_{50} value was defined as the amount of antioxidant necessary to inhibit DPPH radical formation by 50%.

Antioxidant Assay Using the β-Carotene Bleaching Method

The ability of COLF-EO to prevent bleaching of β-carotene was assessed as described by Koleva *et al*.[21] A stock solution of β-carotene/linoleic acid mixture was prepared by dissolving 0.5 mg of β-carotene, 25 µL of linoleic acid and 200 µL of Tween 40 in 1 mL of chloroform. The chloroform was completely evaporated under vacuum in a rotatory evaporator at 40°C, then 100 mL of bi-distilled water was added and the resulting mixture was vigorously stirred. The emulsion obtained was freshly prepared before each experiment. Aliquots (2.5 mL) of the β-carotene/linoleic acid emulsion were transferred to test tubes containing 0.5 mL of essential oil solutions prepared at different concentrations (0.1 to 1 mg/mL). Following incubation for 2 h at 50°C, the absorbance of each sample was measured at 470 nm. BHA was used as a positive standard. A control consisted of 0.5 mL of distilled water, instead of the sample. The antioxidant activity of essential oil was evaluated in terms of the bleaching of β- carotene using the following formula:

$$\textit{Antioxidant activity \%} = [1 - (A_0 - A_t)/ (A_0' - A_t')] \times 100$$

Where A_0 and A_0' are the absorbance of the sample and the control, respectively, measured at time zero, and A_t and A_t' are the absorbance of the sample and the control, respectively, measured after 2-h incubation.

Antimicrobial activity of COLF-EO

Antimicrobial activity assays were performed according to the method described by Berghe and Vlietinck.[22] The antibacterial activities of COLF-EO were tested in duplicate against 11 strains of pathogenic strains: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus thuringiensis, Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus luteus* (ATCC 4698), *Klebsiella pneumoniae* (ATCC 13883), *Enterobacter* sp and *Actinomyces* sp. Solid Luria Broth media (tryptone; 10 g/l, NaCl; 10 g/l, yeast extract; 5 g/l and agar agar; 18 g/l) were inoculated with 100 µl (10^6 UFC/ml) of each tested strain. Three Whatman paper discs (5 mm) were aseptically placed on each plate; 2 of them were tested with 7 mg (10 µl) and 14 mg (20 µl) COLF-EO, respectively, and the third one was used as positive control with either 0.25 mg ampicillin (*E. coli, B. subtilis, S. aureus, E. faecalis, M. luteus, E.* sp *and A.* sp.) or 0.25 mg ciprofloxacin (*B. thuringiensis, K. pneumoniae, S. typhimurium, P. aeruginosa*). All plates were incubated overnight at 37°C. Antibacterial activities were measured as the diameter of the clear zone of growth inhibition, measured using Image J software, and compared to the positive control.

Mutagenic and antimutagenic activities

Viability assays and determination of test concentrations

Cytotoxic doses of the essential oil were determined according to Miyazawa *et al*.[23] The toxicity of the essential oil toward *S. typhimurium* TA98 and *S. typhimurium* TA100 was determined as described by Santana-Rios *et al*.[24]

Mutagenicity and antimutagenicity tests

Mutagenicity and antimutagenicity of COLF-EO were examined using the plate incorporation method[25] described in detail by Sarac and Sen.[26] Known mutagens 4-nitro-o-phenylenediamine (4-NPD) (3 µg/plate) and sodium azide (NaN_3) (8 µg/plate) were used as positive controls for *S. typhimurium* TA98 and *S. typhimurium* TA100, respectively. DMSO/water (1:9, v/v) and ethanol/water (1:1, v/v) were used as a negative control for essential oil. COLF-EO was prepared at concentrations of 0.125, 0.0125 and 0.00125 mg/plate. Mutagenicity inhibition (%) was calculated using the following equation:

$$\textit{Mutagenicity inhibition (\%)} = [(M - S_1) - (M - S_0)] \times 100$$

Where M=number of revertants/plate induced by mutagen alone; S_0=number of spontaneous revertants; and S_1=number of revertants/plate induced by essential oil plus the mutagen.

Antimutagenicity was recorded as follows: strong: 40% or more inhibition; moderate: 25–40% inhibition; low/none: 25% or less inhibition (Negi *et al*., 2003 and Evandri *et al.,* 2005).

Cell proliferation assay

Cell lines and culture conditions

Four continuous human cell lines were investigated for cytotoxicity effect of COLF-EO; Human breast cancer cells (MCF7), mouse melanoma cells (B16), cervical cancer line (HeLa) and human lung cancer cells (LoVo). The cultures were maintained in a humidified 5% CO_2 incubator at 37°C.

Antiproliferation assay

The proliferation rates of four cell lines MCF7, B16, HeLa and LoVo after treatment with COLF-EO were determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[27] The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water-insoluble blue compound formazan, depending on the viability of cells. Briefly, MCF7, B16, HeLa and LoVo cells were plated at 9×10^3/mL in 96-well plates with growth medium to obtain a final volume and incubated for 24 h at 37°C with CO_2 in a humidified atmosphere. Cells were permitted to adhere to the wells for 24 h, and then treated with 10 µL fresh growth medium containing different concentrations of samples (initially dissolved in DMSO, and diluted with growth medium to different concentrations) were added. After incubation for 72 h at 37°C, the medium in each well was collected and washed by phosphate buffered saline (PBS) twice. To determine the cell viability, 10 µL of MTT (5 mg/mL) in PBS was added to each well, and cells were cultured in additional incubation for 4 h. Then, the growth medium and MTT reagent were removed, and DMSO (100 µL) was added to each well and then shaken. After incubation at 37°C for 10 min, absorbance in the control (treated with 0.1% DMSO) and in the sample-treated cells was measured using an enzyme-linked immunosorbent assay (ELISA) reader at 570 nm. Growth inhibition rate was calculated as a percentage as follows:

Where $OD_{control}$ is the absorbance of the control reaction and OD_{sample} is the absorbance of the sample reaction. The experiment was performed in triplicate and mean values were recorded.

IC_{50} (concentration resulting in 50% inhibition) on the cell lines was estimated by linear regression from a plot of percent of the cytotoxicity against the tested concentration.

Statistical analysis

Experimental results concerning this study were expressed as means ± standard deviation of three parallel measurements. The significance of difference was calculated by Student's t test and values $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The yield of essential oil COLF obtained by hydrodistillation of dry material was 0.63% (w/w). The oil had a green-yellow colour, with a strong perfumery odour. Physical analysis of COLF-EO showed a refractive index of 1.528 and a density of 0.972 at 25°C. The COLF-EO was analysed by GC–MS. The individual identified components, with their relative percentages, are given in Table 1. Twenty seven different components, representing about 91.2% of the total oil, were identified. The analyses revealed a complex mixture of the COLF-EO consisting mainly of hydrocarbon, terpene, sesquiterpene, esters, fatty acids, alcohol, ketone and aldehyde compounds. Fourteen major detected components were found to be Nonadecane (21.68%), Heneicosane (10.04%), α-Phellandrene (9.08%), Geranyl isobutyrate (7.83%), Cyclohexane (6.56%), Hexadecanoic acid (5.83%), Octadecanoic acid (4.97%), Hexenyl benzoic acid (3.81%), Phenyl ethyl tiglate (2.76%), α-Pinene (2.34%), Sabinene (1.32%), Camphene (1.19%), Nerolidol (1.09%) and Heneicosane (1.04%). The minor components (<1%) were identified as Myristic alcohol (0.97%), *2*-Hexanone (0.93%), Geranyl propionate (0.51%), Benzaldehyde (0.49%), Isoamyl butyrate (0.43%), Hexanoic acid methyl ester (0.39%), Methyl Tiglate (0.32%) and β-Myrcene (0.31%).

As reported in the literature, many factors, such as the geographical origin, the genetic factors, the plant material and the season in which the plants were collected, may be responsible for the variation of the chemical composition of the essential.[28] To the best of our knowledge no reports describes the chemical composition of essential oil of *C. olitorius* L. species.

Antioxidant activity

General

In this study, two antioxidant assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and β-carotene assay were employed to evaluate the antioxidant activity of COLF-EO.

Table 1: Chemical composition of essential oil isolated by hydrodistillation from *Corchorus olitorius* L. flowers and leaf[a]

Component[b]	Rt (min)[c]	(%)[d]
Hydrocarbons	-	52.09
α-Phellandrene	55.40	9.08
β-Cedrene	55.79	0.32
Cyclohexane	59.35	6.56
α-Pinene	68.49	2.34
Nonadecane	69.73	21.68
Heneicosane	74.56	1.04
α-Terpinene	79.18	10.04
Terpenes	-	2.09
Limonene	17.90	0.90
Camphene	25.38	1.19
Germancrene D	31.57	2.87
Terpene	-	2.72
Nerolidol	51.81	1.09
β-Myrcene	56.23	0.31
Sabinene	60.67	1.32
Esters	-	13.82
Methyl Tiglate	9.77	0.32
Hexanoic acid, methyl ester	12.16	0.39
Isoamyl butyrate	16.78	0.43
Geranyl isobutyrate	48.79	7.83
Phenyl ethyl tiglate	53.17	2.76
Geranyl propionate	60.41	0.51
1,2-Benzenedicarboxylic acid, dibutyl ester	72.93	1.5
Aldehyde	-	0.49
Benzaldehyde	14.12	0.49
Fraxinellone	-	0.93
2-Hexanone	7.37	0.93
Alcohol	-	0.97
Myristic alcohol	7.48	0.97
Fatty acids	-	14.61
Hexenyl benzoic acid	53.68	3.81
Hexadecanoic acid	71.04	5.83
Octadecanoic acid	80.44	4.97
1,2-Benzenedicarboxylic acid	82.32	1.37
Identification components (%)	-	91.20
Undefined compound (%)	-	8.80

[a]Data are the means of two replicates ; [b]Identification of components based on GC–MS Wiely 7.0 version library ; [c]Retention time ; [d]Percentages are the means of two runs and were obtained from electronic integration measurements using a selective mass detector.

DPPH free radical-scavenging activity

DPPH scavenging assay is based on a measurement of discoloration resulting from a reduction of DPPH free radical by an antioxidant.[20] The decrease in absorbance is taken as a measure of radical-scavenging activity. This is a widely used method to investigate the scavenging activity of some natural compounds. As can be seen in Figure 1, the scavenging activity of COLF-EO is concentration-dependent. The IC_{50} value of the essential oil is 0.49 mg/mL, whereas the IC_{50} of BHA is 11μg/mL. The free radical-scavenging activity of COLF-EO was higher than that of *Marrubium globosum* essential oil which had an IC_{50} value of 1.20 mg/ml.[29] However, it is lower compared to other essential oils extracted from *Marrubium vulgare*[30] and *Teucrium marum*[31] which showed IC_{50} values corresponding respectively to 0.074 and 0.013 mg/mL, respectively. The obtained results here in were found to be in agreement with the findings of several authors who reported that the efficiency of an antioxidant component to reduce DPPH essentially depends on its hydrogen donating ability, which is directly related to the presence of phenolic compounds, the abundance of monoterpenes hydrocarbons and oxygenated monoterpenes.[32] Table 1 shows that essential oil of COLF is notably rich in non-phenolic components.

Antioxidant activity measured by the β-carotene bleaching assay

The β carotene–linoleic bleaching inhibition assay simulates membrane lipid oxidation and can be considered a good model for membrane-based lipid peroxidation. In this oil–water emulsion-based system, linoleic acid acts as a free radical generator that produces peroxyl radicals under thermally induced oxidation. These free radicals attack the β-carotene chromophore resulting in a bleaching effect, which can be inhibited by a free-radical scavenger.[33] The potential of COLF-EO to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test. The results of antioxidant activities of COLF-EO and BHA are presented in Figure 2. At the same concentration (0.4 mg/mL), the antioxidant activity of the essential oil was twofold lower than the activity achieved by BHA (48.50 ± 2.8% versus 96 ± 0.5%). Moreover, at higher concentration used (1.0 mg/mL), the potential of essential oil sample to inhibit lipid peroxidation was improved to 77.12 ± 0.7% versus 97 ± 2.1% for the positive control. This antioxidant activity was more important than *Eruca sativa L.* flowers of which the essential oil showed 75% inhibition at 50 mg/mL.[34]

Antibacterial activity

In vitro antibacterial activities of COLF-EO were tested against 11 pathogenic strains (7 Gram + and 4 Gram -) as listed in the experimental section. The inhibition zones, measured in mm, qualitatively and quantitatively assessed all activities. The obtained results (Figure 3, Table 2) show that COLF-EO exhibits potent inhibitory effects against both Gram + and Gram–strains and even more efficient than the antibiotic ampicillin tested against the strains *B. thuringiensis, K. pneumoniae, S. typhimurium* and *P. aeruginosa.* The diameter of the inhibition zones (Table 2) was between 12.5 ± 2.1 mm and 16.7 ± 0.1 mm, when using just 6 mg of COLf-EO.

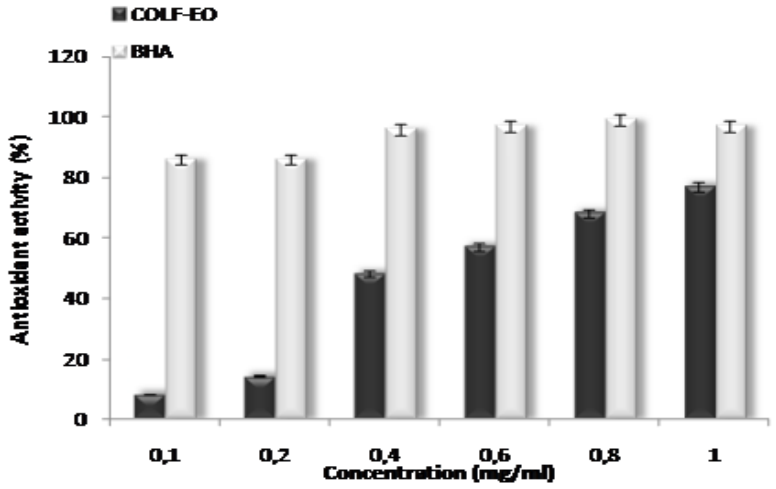

Figure 1: Free radical-scavenging capacities of COLF essential oil and BHA as positive control measured by DPPH assay

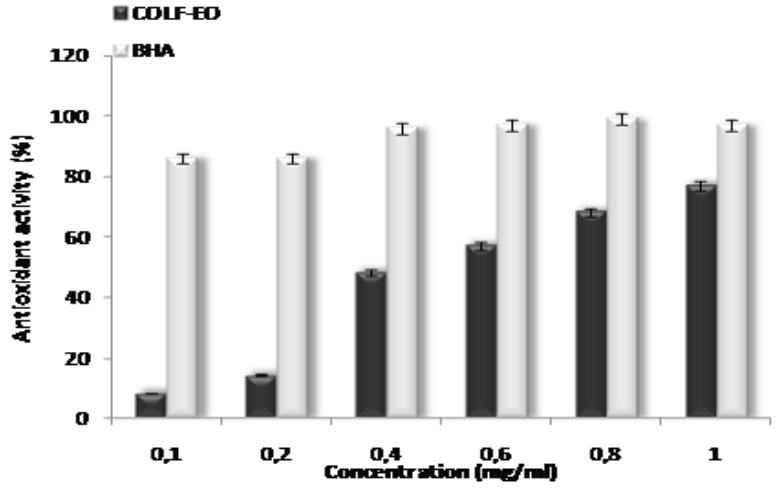

Figure 2: Antioxidant activities of COLF essential oil and BHA as positive control measured by β-carotene bleaching method

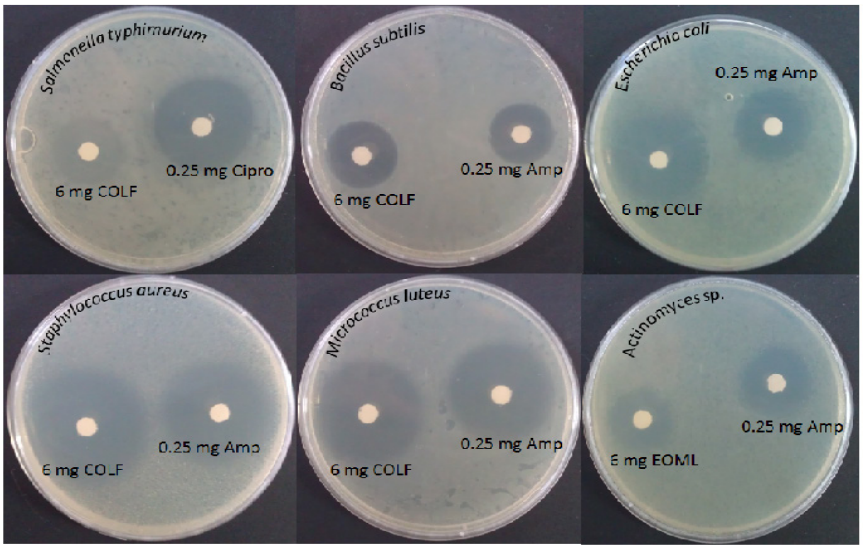

Figure 3: Some antibacterial activities of essential oil from *Corchorius olitorius* flowers and leaf (COLF). Both inhibitions against Gram + and Gram- bacteria (*E. coli*, *B. subtilis*, *S. aureus*, *S. typhimurim*, *M. luteus*, and *A. sp.*) were evaluated. Amp; Ampicillin

Table 2: Diameter of the inhibition zones of bacterial growth using solvent extract from rocket Àowers (COLF). Gram+ and Gram- bacteria were tested; ampicillin and ciprofloxacin were used as positive controls. N.D. not determined

Bacterial strains		Antibiotics		COLF sample
		0.25 mg Ampicillin (mm)	0.25 mg Ciprofloxacin (mm)	6 mg COLF (mm)
Gram +	*Bacillus subtilis*	24.6 ± 0.9	N.D.	16.6 ± 1.3
	Staphylococcus aureus	27.7 ± 3.4	N.D.	12.6 ± 0.9
	Enterococcus faecalis	33.5 ± 2.9	N.D.	14.9 ± 2.3
	Bacillus thuringiensis	0.0	22.3 ± 2	15.6 ± 0.5
	Micrococcus luteus	33.3 ± 1.8	N.D.	14.1 ± 2.1
Gram -	*Klebsiella pneumoniae*	0.0	25.4 ± 2.4	14.8 ± 1.5
	Actinomyces sp.	27.4 ± 2.1	N.D.	12.6 ± 2.9
	Escherichia coli	35.7 ± 0.9	N.D	16.0 ± 1.0
	Salmonella typhimurium	0.0	20.1 ± 2.6	16.7 ± 0.1
	Enterobacter sp.	23.2 ± 1.6	N.D.	13.3 ± 2.2
	Pseudomonas aeruginosa	0.0	24.6 ± 2.4	12.5 ± 2.1

Table 3. Results of antimutagenicity assays of essential oil from *Corchorus olitorius* L. flowers and leaf for *S. typhimurium* TA98 and TA100 bacterial strains

Test items	Concentration (mg/plate)	Number of revertants			
		TA98		TA100	
		Mean ± S. error	Inhibition%	Mean ± S. error	Inhibition%
Negative control	-	9.33 ± 4.5[b]	-	9.33 ± 2.51	-
4-NPD[a]	3	196 ± 14.84	-	-	-
NaN_3[a]	8	-	-	356 ± 15.57	-
COLF-EO	0.125	58.2 ± 4.86	70.31	98.8 ± 5.49	72.25
	0.0125	66.2 ± 2.77	66.22	117.2 ± 3.19	67.08
	0.00125	72.2 ± 3.76	63.16	140.4 ± 5.68	60.56

[a]4-NPD and NaN_3 were used as positive controls for *S. typhimurium* TA98 and TA100 strains, respectively ; [b]Values expressed are means ± SD of three replications. Regression analysis for mutagenicity inhibition (%) and plant extract concentrations (log values) (R^2: 0.99) was performed using Microsoft Excel.

The growth inhibition was dependent on extract concentration. The sensibility to the COLF-EO was found to differ among the test microorganisms. In fact, Gram positive bacteria were the most sensitive being inhibited by the essential oil. These differences could be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram negative bacteria compared to the single membrane structure of positive ones.[35] The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action. They were dependent of crucial extracellular microbial enzymes inhibition, growth inhibition by substrate deprivation or acting on direct metabolism through oxidative phosphorylation inhibition.[36]

The microorganisms tested in the present investigation are large and cover the most important human pathogens known as opportunists for man and animals and cause food contamination and deterioration. In fact, essential oil could interact with the cell membrane and accumulate in the lipid bilayer of bacteria occupying a space between the chains of fatty acids.[37] The strong antimicrobial activity of the COLF-EO against the tested microorganisms could be attributed to the presence of high percentage of hydrocarbon (52.09), monoterpene (2.9), and oxygenated monoterpene (2.09) appreciated for their antibacterial potentials (28 and Yangui *et al.*, 2009). In addition to the hydrocarbon components of COLF-EO, Camphor may be the main compounds responsible for the antimicrobial effect of the essential oil.[38] These results are in agreement with those reported in the literature for other essential oils rich in camphor such as *Artemisia herba-alba* oil which showed a very strong action versus *S. aureus*, *Candida tropicalis* and *Candida albicans*.[39]

Antimutagenic activities

This study examined also the potential antimutagenic activity of COLF-EO toward *S. typhimurium* TA98 and TA100. The findings are summarized in Table 3. The antioxidant properties of phytochemicals are linked to their ability to scavenge free radicals generated either endogenously or by exogenous agents. These preventive agents can inhibit the

Table 4: Cytotoxic activity of the essential oil isolated from *Corchorus olitorius* L. flowers and leaf

Cell lines	IC_{50} (µg/mL) a
Hela	4 ± 0.98
MCF7	15 ± 1.40
B16	57 ± 1.68
LoVo	26 ± 0.83

[a]IC_{50} values expressed are means±S.D. of three parallel measurements.

mutation and cancer initiation process by modulating phase I and phase II enzymes, by blocking reactive species either by scavenging, electron donation or through chelation and thus maintains the DNA structure. This study used the Ames *Salmonella*/microsome mutagenicity test, a standard plate incorporation method, to identify antimutagenic activity of COLF- EO. AMES test results indicated strong antimutagenic activity of the COLF-essential oil at all of the concentrations tested and were found to be dose-dependent. Moreover, the strongest antimutagenic activity was observed in the 0.125 mg/plate concentration of the tested essential oil against *S. typhimurium* TA100. Also the COLF-EO did not exhibit any mutagenicity at any of the concentrations tested. Therefore, it is interesting to investigate the effect of COLF-EO in inhibiting mutagenicity *in vivo* studies. The present results showed the antimutagenic activity in Ames test that may be attributed in part to powerful radical scavenger associated with the essential oil. Negi *et al*,[40] a compound is found to possess its less antimutagenic activity if its percentage of inhibition is less than 25%, a moderate activity if the percentage inhibition value lies between 25% and 40% and a strong antimutagenicity effect if it is more than 40%. COLF-EO reduces the mutagenicity by 97.21% and 90.30% respectively in the strains TA98 and TA100 at the highest tested dose (0.125 mg/plate) which shows strong antimutagenic activity. The inhibition of mutagenesis are grouped into two namely desmutagens and bioantimutagens. Antimutagenic effects of EOs may be confined to their ability to inhibit penetration of mutagens inside the cells, free radical scavenging activity, activation of antioxidant enzymes[41-42] and inhibition of P450 mediated formation of mutagens.[43] Interference with mutation inducing DNA repair systems[44] and induction of necrosis/apoptosis leading to cellular death are among the proposed mechanisms behind antimutagenic activity of essential oils.[45]

Cytotoxicity assay

One of the most difficult challenges in chemotherapy is treatment of malignant cell growth leading to cancer. The cytotoxic activity of COLF-EO was examined using four mammalian cancer cell lines mentioned above. The inhibition of the proliferation of cancer cells in the presence of COLF-EO occurred in a concentration-dependent manner. As shown in Table 4, the growth inhibition effect of the EO on HeLa cell line (IC_{50}= 4 ± 0.98 µg/mL) was stronger than on other cell lines. The B16 cell line exhibited the lowest sensitivity to the EO with an IC_{50}>50 µg/mL. Cytotoxic activity of the essential oil is probably related to the presence of phenols, aldehydes and alcohols, which was already reported by Lei *et al*.[46] Because of the great number of constituents, EO seems to have no specific cellular targets.[47] As typical lipophiles, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Cytotoxicity appears to include such membrane damage. Generally, the cytotoxicity of essential oil in mammalian cells is caused by induction of apoptosis and necrosis.[45] Furthermore, essential oils are reported to interfere with membrane functions, ion homeostasis as well as cell signalling events of cancer cell lines. It is found to inhibit DNA synthesis and reduce the size of colon tumours.[48] In addition, essential oils exhibit capacity to act as antioxidants and interfere with mitochondrial functions of mammalian cells. As a result, EOs diminish metabolic events (for example, increased cellular metabolism, mitochondrial overproduction and permanent oxidative stress) characteristic of malignant tumour development.[49]

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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