ORIGINAL ARTICLE

BIOLOGICAL ACTIVITY OF *CURCUMA LONGA* AND *ORIGANUM MARJORANA*, CULTIVATED IN EGYPT

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ABSTRACT

Background, medicinal plants have been used as a source of therapies since ancient times in Egypt. **Objectives**, the present study was designed to investigate the antibacterial, antioxidant and antitumor activity of aqueous, ethanol and acetone extracts from *Curcuma longa* and *Origanum marjorana* cultivated in Egypt. The study was also extended to identify the phenolic compounds present using HPLC then evaluate their antibacterial and antitumor activity of the most abundant compounds. **Methods**, the disk diffusion method and micro-broth dilution were used to determine MIC and MBC of the plant extracts against ten bacterial strains belonging to five species, *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes*. Phytochemical screening followed by 2,2-diphenyl-1-picrylhydrazyl assay were used to assess the antioxidant of the extracts then the large intestinal carcinoma (CACO) cell line was used to evaluate antitumor of these extracts by Sulfo-Rhodamine B colorimetric (SRB) assay. **Results**, the results indicated that studied crude extracts were able to inhibit the growth of at least 5 of the tested bacteria. The two studied plants have various bioactive components and showed moderate antioxidant activity, moreover the results show that IC_{50} values below 20 µg/mL were recorded for the crude extract of both tested plants. Rutin, benzoic acid and salicylic acid were the most abundant phenolic compounds in *C. longa* and *O. marjorana*. Rutin and benzoic acid showed anti-bacterial activity against 100% and 80% of tested bacteria, respectively and also expressed moderate cytotoxic activity with IC_{50} 22.7 and 47.8 µg/mL, respectively. **Conclusions,** the present investigation provided supportive data for the possible use of the plant extracts investigated here in treatment of various diseases.
***Keywords**: Antibacterial, Antioxidant, Cytotoxicity, HPLC, Medicinal plants, Phyto-chemicals.*

1. INTRODUCTION

Herbal medicines are often used to provide first line and basic health service to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Even in areas where modern medicine is available, the interest on herbal medicines has been increasing rapidly in recent years. These medicines are relatively safer and cheaper than synthetic or modern medicine [1]. The World Health Organization has estimated that 80% inhabitants of the world especially those of developing countries such as Egypt, India and China, rely chiefly on traditional medicines for their basic preventive and curative healthcare [2]. *C. longa* is a widely used as a spice for flavor and color in food preparations, and has shown valuable effects on health, mostly for its antioxidant properties. These activities have been qualified to phenolic compounds present in its rhizomes. Many studies have demonstrated antioxidant, anti-inflammatory and antimicrobial activity for *C. longa* [3]. In folk medicine, *O. majorana* is used to treat asthma, indigestion, cramps, headache, depression, and rheumatism, and it has diuretic activity. This plant has a strong antioxidant activity attributed to its high content of flavonoids, phenolic acids and essential oils which have shown antiviral, antibacterial and antifungal activity [4,5].

Bacterial diseases is a type of infectious diseases caused by pathogenic bacteria which have always been considered as a major cause of morbidity and mortality in humans. Resistance to antibiotics is one of the biggest problems that face public health [6]. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs because of their lesser side effects and low resistance in microorganisms [7,8].

Plants generally produce biologically active compounds "Phytochemicals" as polyphenols which have been reported to have multiple biological properties such as antioxidant, antimutagenic, antibacterial, antiviral, and anti-inflammatory activities [9]. These Phytochemicals are generally obtained from plant materials by steam distillation

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or by extraction with organic or aqueous solvents [10]. The aim of this study is to investigate the antibacterial, antioxidant, and anti-cancer effect" of the aqueous and organic extracts of *C. longa* and *O. marjorana* cultivated in Egypt.

2. MATERIALS AND METHODS

2.1. Plant Samples and Extraction

Fresh samples of *C. longa* and *O. marjorana* were collected from a local market in Cairo, Egypt then dried at 50ºC and reduced to fine particles using Waring laboratory blender (MX-7011G) for 5 min at high speed and then stored in airtight closed bottles for two days before being used for analysis. For aqueous extracts, 300 g dried ground plant material was soaked in 1L distilled water then heated in water bath at 40ºC for 2 hrs and for organic extract, 300 gm plant material was percolated with 1L ethanol or 1 L acetone in glass bottles, those bottles were vigorously shaken at a speed of 300 rpm, overnight. Plant residues were allowed to settle and the supernatant was filtered. All filtrates obtained were concentrated under reduced pressure at 68°C in a rotary evaporator to obtain the crude extracts which were kept at 4°C until further uses. The percentage yield of extracts for different solvents was calculated using the formula: Weight of final extract/ Weight of powder obtained X100.

2.2. Antibacterial Activity:

2.2.1. Microorganisms: The tested organisms for *in-vitro* antibacterial screening were five reference microorganisms "*P. aeruginosa* (NCTC 10662), *K. pneumonia* (ATCC 10031), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 12344), and five clinical isolated multidrug resistant bacteria "MβL, *P. aeruginosa,* ESβL, *K. pneumonia*, ESβL, *E. coli,* MRSA, *S. pyogenes* which were isolated and identified by automated biochemical tests using Vitek®MS colorimetric identification card (bioMerieux - Marcy-l'Étoile, France). The susceptibility patterns were obtained using Vitek®MS aspartate amino-transferase. One single colony of each tested microorganism, taken from nutrient agar stock cultures into 10 mL sterile Muller-Hinton broth medium then incubated at 37°C for 16 - 20 hrs.

2.2.2. Disc Diffusion Assay [11]: 10 µl of each plant extract (100 mg/mL), prepared by dissolving of 100 mg of crude extract in 1 mL distilled water in case of aqueous extracts and 1 mL dimethyl sulfoxide in case of organic extracts, was applied to each filter paper disc (6 mm diameter) to give a final amount of 1 mg plant extract per disc. The discs were air dried and placed on top of the inoculated agar layer and incubated for 16-20 hrs at 37°C. Standard antibiotic discs served as positive control. All antimicrobial studies were done in triplicates.

2.2.3. Determination Of Minimum Inhibitory Concentration (MIC) And Minimum Bactericidal Concentration MBC [12]: Serial dilution of each extract that showed significant zones of inhibition were individually placed in tubes labeled 1 to 10, each tube was contained 1 mL M-H broth inoculated with bacterial suspension. The resulting mixtures were incubated at 37°C for 24 hrs. Turbidity was measured, and the lowest concentrations which did not show any turbidity was determined as minimum inhibition concentration. In order to determine minimum bactericidal concentration values, 100 µL of the content of the tubes with no turbidity were cultured on the M-H agar medium and incubated at 37°C for 24 hrs.

2.3. Qualitative Phytochemical Analysis: Presence or absence of Flavonoids, Alkaloids, Glycosids, Terpenes, Phenolics, Saponins, Tannins were determined using regular protocol as described by Ashfaq et al. (2012) [13].

2.4. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay [14]: The antioxidant activity of plant material was assayed by DPPH assay. 10 µl of plant extract (1mg/mL) was added to 100 µL of 0.2 mM DPPH solution in a microtitre plate. The reaction mixture was incubated at 25ºC for 5 min, then measured at 520 nm. The DPPH without plant material served as control. Methanol with respective plant extracts serves as blank. The percent DPPH scavenging activity was calculated as: $[(A_B - A_T) / A_B] \times 100$. Where A_B and A_T are the absorbance of blank and plant material, respectively.

2.5. Potential Anticancer Assay:

2.5.1. Cell Culture: CACO cell line (86010202, Sigma Ald.) was used, starting from a frozen ampoule, add thawed cells to 4 mL of RPMI 1640 medium (R8758, Sigma Ald.) in the tube. Centrifuge the cell suspension at low speed for 5 min. The media was removed and re-suspend the cell pellet at a density of 1 x 10^4 cells/mL in fresh medium supplemented with 10% FBS "fetal bovine serum" (v/v) and 100 mg/mL streptomycin and 100 IU/mL penicillin at 37°C in a CO_2 incubator with 5% CO_2.

2.5.2. SRB (Sulfo-Rhodamine B colorimetric) Assay [15]: This assay has been used for high-throughput drug screening at the National Cancer Institute. The plant extracts under test were used to prepare stock solutions (1 mg/mL) using DMSO. Different concentrations (0.0, 12.5, 25.0, 50.0 and 100.0) µg/mL of each stock was prepared by serial dilution using the medium. 100 µL of cells (1×10^4 cells/mL) was seeded in 96-well plates and incubated at 37◦C, 5% carbon dioxide. After 24 hrs of incubation, the cells were treated with 100 µL of plant extracts. Plates were incubated at 37°C, 5% CO_2. After 48 hrs incubation, cells were washed and stained with twenty microliters of SRB stain. The plates were further incubated for 4 hrs and excess stain was washed with 1% acetic acid, then attached stain was recovered by "Tris EDTA" buffer. The wells with only culture medium treated with 1% of DMSO served as control. The absorbance was measured at 560 nm for each well on an absorbance plate reader. The relation between surviving fraction and extract conc. was plotted to get the survival curve. Survival fraction (S.F.) = Absorbance $_{sample}$ / Absorbance $_{control}$. Then the half maximal inhibitory concentration (IC_{50}) was calculated for each extract after 24 hrs of exposure from survival curve. IC_{50} indicates the lowest concentration of plant extracts that inhibits 50% of cells.

2.6. HPLC For Phenolic Compounds In Plant Extract

2.6.1. Sample And Standard Compounds Preparation: Using ethanol HPLC spectral grade, 10 mg/mL of plant extracts and 10 µg/mL of sixteen different pure known phenolic compounds as external standards (gallic acid, catechol, *p*- hydroxy benzoic acid, caffeine, vanillic acid, caffeic acid, syringic acid, vanillin, *p*- coumaric acid, ferulic acid, rutin, ellagic acid, benzoic acid, *o*- coumaric acid, salicylic acid and cinnamic acid were prepared before injection in the analytical HPLC system and chromatographed. Prior to HPLC analysis, all solutions (samples and standards) were filtered through 0.2 µm syringe filter.

2.6.2. HPLC System: Chromatographic analysis were carried out using Agilent 1260 Infinity HPLC Series (Agilent, USA), equipped with Quaternary Pump (G1311B), Autosampler (G1329B, ALS) and Diode Array Detector G1315D, VL) coupled to Agilent Open LAB ChemStation B.04.03 software. Phenolic compounds were separated on a ZORBAX Eclipse plus C18 reversed-phase column (100 mm x 4.6 mm, 5 µm) (Agilent technologies, USA).

2.6.3. Chromatographic Conditions [16]: At 25°C, The separation is achieved using a ternary linear elution gradient with solvent A (HPLC grade water and 0.2 % H_3PO4 (v/v) at pH 2.65), solvent B (methanol) and solvent C (acetonitrile) with flow rate 0.7 mL/min. The injected volume of plant extract was 20 µL. VWD detector set at 284 nm. The concentration of an individual compound was calculated on the basis of peak area measurements.

2.6.4. HPLC Analysis And Identification Of Compounds: Based on a combination of retention time (Rt) and spectral matching with those of pure standard, the results were expressed as area % of each identified compound from the total area.

2.7. Screening Of Antibacterial And Cytotoxicity Of Some Identified Phytochemicals: Based on the results obtained by HPLC assay, some phenolic compounds, which were present in high concentration, were selected for evaluation of their antibacterial and antitumor activities. The stock solution of selected compounds in concentration of 50 mg/mL were prepared by dissolving of 50 mg of these phenols in 1 mL sterile distilled water. The antibacterial activity, MIC, MBC and antitumor activity were applied according to the methods mentioned before.

2.8. Statistical Analysis: The data were expressed as standard error of the mean and comparisons between groups were performed with paired students t-test on a Statistical Software Package (SPSS). Differences were considered significant, if p value is less than 0.05 "$p < 0.05$".

3. RESULTS

The average percentage of extraction yields with ethanol was better than that obtained by acetone or distilled water (Fig 1).

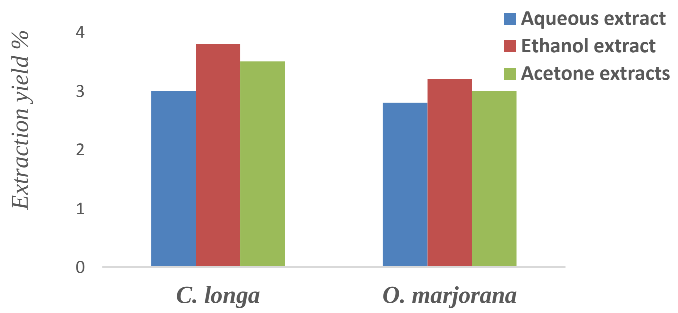


Figure 1: The figure presents extraction yields in %.

Table 1. The table presents the antibacterial activities (diameter of inhibition zone, mm) of *C. longa* and *O. marjorana* against tested bacteria.

Plant extracts		Used bacteria / Mean diameter of inhibition zone in mm (mean ± SD)									
		Reference strains					*Clinical isolates*				
		P. aeruginosa NCTC 10662	*K. pneumonia* ATCC 10031	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*S. pyogenes* ATCC 12344	MβL, *P. aeruginosa*	ESβL, *K. pneumonia*	ESβL, *E. coli*	MRSA	*S. pyogenes*
C. longa	aq	7.17 ± 0.24	7.00 ± 0.00	8.33 ± 0.24	10.50 ± 0.41	8.33 ± 0.47	-		-	7.17 ± 0.24	-
	eth	11.00 ± 0.62	11.17 ± 0.85	11.00 ± 0.82	13.00 ± 0.00	7.00 ± 0.00	8.8 ± 0.62	-	10.5 ± 0.24	10.0 ± 0.24	8.00 ± 0.00
	ace	8.33 ± 0.47	8.83 ± 0.62	7.83 ± 0.62	9.33 ± 0.47	-	8.00 ± 0.00		7.50 ± 0.41	7.50 ± 0.41	7.50 ± 0.41
O.marjorana	aq	-	-	-	-	-	-	-	-	-	-
	eth	-	7.83 ± 0.62	10.00 ± 0.71	7.83 ± 0.62	-	-	-	9.00 ± 0.82	8.33± 0.24	-
	ace	-	7.67 ± 0.47	-	7.17 ± 0.24	-	-	-	-	7.17± 0.24	-
TE		21.83 ± 0.62	16.17 ± 0.62	18.83 ± 0.62	20.33 ± 0.47	16.83 ± 0.62	18.50 ± 0.41	16.83 ± 0.62	17.83 ± 0.62	18.67 ± 0.47	-
AMP		12.0 ± 0.41	-	15.50 ± 0.41	25.67 ± 0.47	20.33 ± 0.47	20.33 ± 0.47	-	-	-	-
E		-	-	-	21.67 ± 0.47	25.67 ± 0.47	-	-	-	17.83 ± 0.62	-
OFX		24.00 ± 0.00	14.33 ± 0.47	29.50 ± 0.41	24.33 ± 0.47	21.83 ± 0.62	16.83 ± 0.62	14.33 ± 0.47	19.33 ± 0.47	20.33 ± 0.47	22.17 ± 0.24
VA		-	-	-	22.50 ± 0.41	20.33 ± 0.47	-	-	-	21.67 ± 0.47	15.50 ± 0.41
CTX		22.17 ± 0.24	18.83 ± 0.62	22.50 ± 0.41	18.50 ± 0.41	16.83 ± 0.62	-	-	-	14.33 ± 0.47	-
CEC		20.33 ± 0.47	20.33 ± 0.47	20.33 ± 0.47	21.83 ± 0.62	22.17 ± 0.24	-	-	-	-	19.33 ± 0.47
DO		24.33 ± 0.47	21.83 ± 0.62	15.50 ± 0.41	25.67 ± 0.47	16.17 ± 0.62	19.50 ± 0.41	19.33 ± 0.47	15.50 ± 0.41	22.50 ± 0.41	-
AK		18.83 ± 0.62	22.17 ± 0.24	29.50 ± 0.41	12.33 ± 0.24	15.50 ± 0.41	18.67 ± 0.47	20.33 ± 0.47	22.17 ± 0.24	13.50 ± 0.41	15.50 ± 0.41
DA		-	-	-	18.83 ± 0.62	22.17 ± 0.24	-	-	-	18.50 ± 0.41	19.50 ± 0.41

- : No inhibition zone; aq: hot water extrac; eth: ethanol extract; ace: acetone extract; ATCC: American Type Culture Collection; NCTC: National Collection of Type Cultures; ESβL *K. pneumonia*: Extended Spectrun β Lactamase producing *K. pneumonia*; ESβL *E. coli*: Extended Spectrun β Lactamase producing *E. coli*; MβL *P. aeruginosa*: Metallo-beta-lactamase producing *P. aeruginosa*; MRSA: Methicillin-resistant *S. aureus*; TE: Tetracyclin 30 µg; AMP: Ampicillin 10 µg; E: Erythromycin 15 µg; OFX: Ofloxacine 10 µg; VA: Vancomycin 30 µg; CTX: Cefotaxime 30 µg; CEC: Cefaclor 30 µg; DO: Doxycycline 30 µg; AK : Amikacin 30 µg; DA: Clindamycin 30 µg.

The results of antibacterial action showed that *C. longa* was stronger antibacterial activity compared to *O. marjorana*. All tested bacteria except ESβL, *K. pneumonia* were inhibited by *C. longa* while *O. marjorana* produced antibacterial activity against only five tested bacteria. In addition, the result of inhibition zone confirmed that the ethanol extract was more effective than aqueous and acetone extracts (Table 1).

Table 2. The table presents MIC and MBC (mg/mL) of tested plant extracts against tested organisms.

Plant extracts		Tested organisms --- (MIC) /(MBC) (mg/mL)									
		P. aeruginosa NCTC 10662	*K..pneumonia* ATCC 10031	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*S. pyogenes* ATCC 12344	MβL, *P. aeruginosa*	ESβL, *K. pneumonia*	ESβL, *E. coli*	MRSA	MDR, *S. pyogenes*
C. longa	aq	12.5/25	25/50	6.25/12.5	6.25/6.25	25/50	-	-	-	12.5/25	-
	eth	6.25/6.25	6.25/6.25	3.13/3.13	12.5/25	25/50	12.5/12.5	-	6.25/6.25	6.25/6.25	25/50
	ace	6.25/12.5	25/25	6.25/12.5	12.5/12.5	-	12.5/25	-	6.25/12.5	12.5/25	50/50
O. marjorana	aq	-	-	-	-	-	-	-	-	-	-
	eth	-	100/200	100/200	100/100	-	-	-	100/200	100/100	-
	ace	-	100/200	-	100/200	-	-	-	-	200/200	-

- : No antibacterial activity; aq: hot water extrac; eth: ethanol extract; ace: acetone extract; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration MDR: multi-drug resistant; ESβL *K. pneumonia*: Extended Spectrun β Lactamase producing *K. pneumonia*; ESβL *E. coli*: Extended Spectrun β Lactamase producing *E. coli*; MβL *P. aeruginosa*: Metallo-beta-lactamase producing *P. aeruginosa*; MRSA: Methicillin-resistant *S. aureus*.

MIC ranged from 3.13 to 200 mg/mL the least was 3.13 mg/mL for *C. longa* against *E. coli* ATCC 25922, the highest was 200 mg/mL for *O. marjorana* against MRSA. The MBC ranged from 3.13 to 200 mg/mL the least was 3.13 mg/mL for *C. longa* against *E. coli* ATCC 25922, the highest was 200 mg/mL for *O. marjorana* against *K. pneumonia* ATCC 10031, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, ESβL, *E. coli*, MRSA (Table 2).

Phytochemical screening proved the presence of flavonoids, phenolics, saponins, tannins etc. in organic extacts of the two plants as shown in Table 3, howerver the water extract of *C. longa* did not extract the flavenodis or phenolic compounds. Moreover, flavenois, alkaloids and tannins of *O. marjorana* were not extacted by aqueous solution.

Table 3. The table presents qualitative chemical analysis of phytoconstituents in different extracts of tested plants.

	Flavonoids			**Alkaloids**			**Glycosids**			**Terpenes**			**Phenolics**			**Carbohydrates**			**Proteins**			**Saponins**			**Tannins**		
	aq	ath	ace	aq	ath	ace	aq	ath	ace	aq	ath	ace	aq	ath	ace	aq	ath	ace	aq	ath	ace	aq	ath	ace	aq	ath	ace
C. longa	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O. marjorana	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+

aq, hot water extract; eth, ethanol extract; ace, acetone extract, + : present; - :absent.

All extracts produced moderate DPPH scavenging activity (< 90 - 40% Inhibition). The highest DPPH activities were observed in ethanol and acetone extracts of *O. marjorana* (87.3 and 84.4, respectively), and in acetone extract of *C. longa* (82%) (Fig 2).

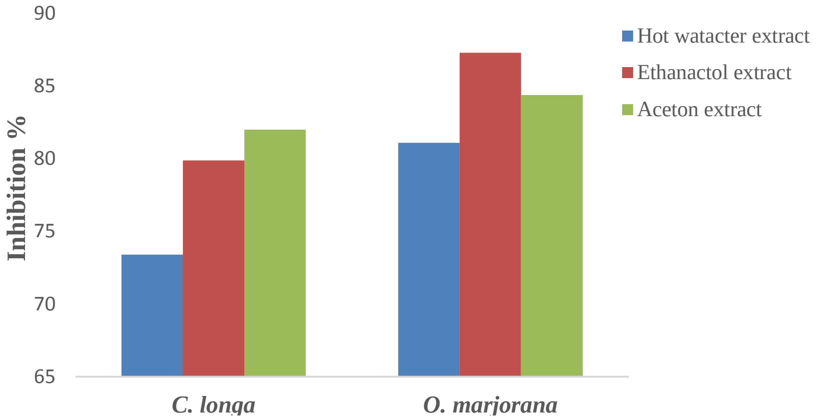


Figure 2. The figure presents DPPH free radical scavenging activity (% Inhibition) of different extracts of tested medicinal plants.

O. marjorana and *C. longa* were able to inhibit the proliferation of CACO cell line (Fig 3) and induced more than 50% inhibition with IC_{50} values 17.0 and 17.7 µg/mL, respectively.

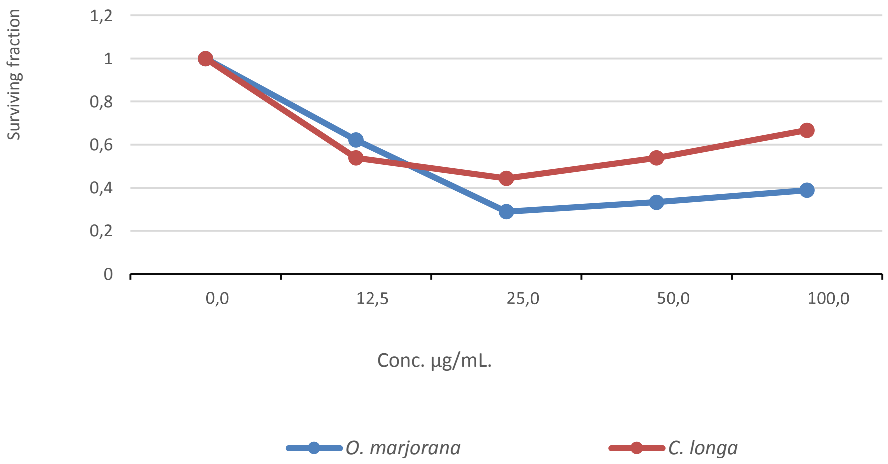


Figure 3. The figure presents potential antitumor assay of plant extracts at different conc. using CACO cell line.

HPLC chromatogram for *C. longa* and *O. marjorana* are presented in Fig 4 and Fig 5, respectively .Table 4 shows the concentrations of detected phenolic compounds expressed in mg/100 g dry sample. *P*- Hydroxy benzoic acid, caffeic acid, vanillin, *p*- coumaric acid, ferulic acid, rutin, ellagic acid, benzoic acid, and salicylic acid were detected in both tested plants, while syringic acid was absent in both. Caffeine, gallic acid, and cinnamic acid were detected in *O. marjorana* in concentrations of 33.2, 3.22 and 5.72 mg/100g DW, respectively, while catechol, vanillic acid, and *o*- coumaric acid have been detected in *C. longa* (1.39, 1.03, and 0.86 mg/100g DW, respectively).

As can be seen, the predominant compounds within *C. longa* were benzoic acid followed by rutin (70.83 and 41.62 mg/100g DW, respectively). These two substances together make up about 76.7 % of total phenolic compounds that were identified in *C. longa*. Benzoic acid, salicylic acid followed by rutin were the major compounds in *O. marjorana* (706.12, 216.0 and 155.0 mg/100 gm DW, respectively) and together make up about 87% of total identified phenolic compounds in *O. marjorana*. Other detected phenolic compounds in both plant extracts were present in low to moderate concentrations. When comparing total content of the identified phenolic compounds in both *C. longa* and *O. marjorana*, it was noticed that *C. longa* contains about 90.9% phenolic compounds less than *O. marjorana*.

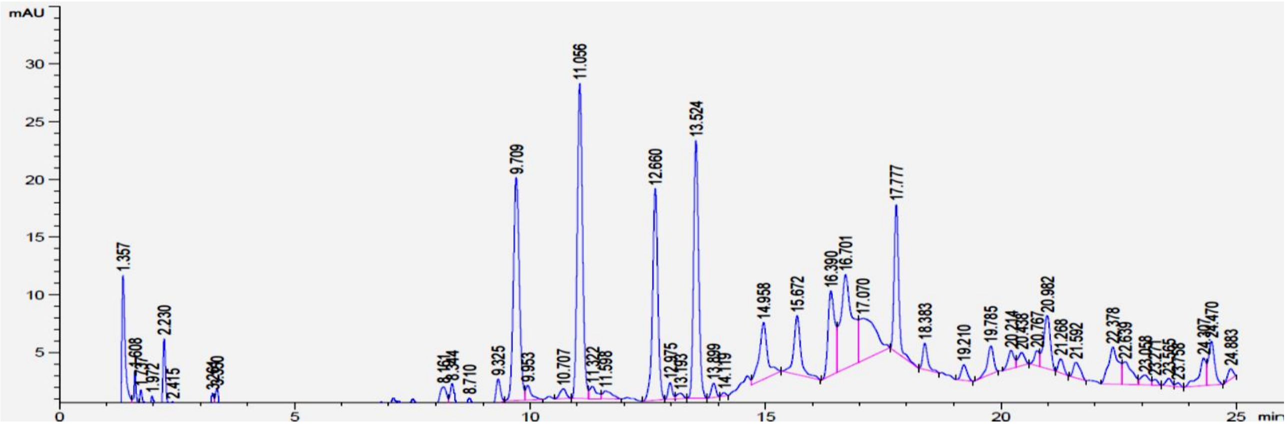


Figure 4. The figure presents HPLC profile of phenolics in *C. longa*.

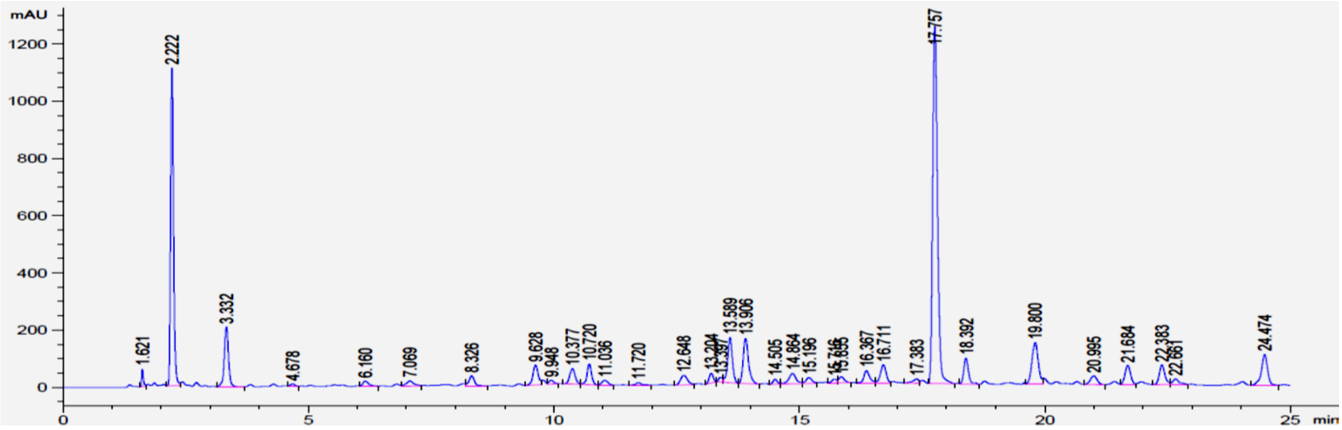
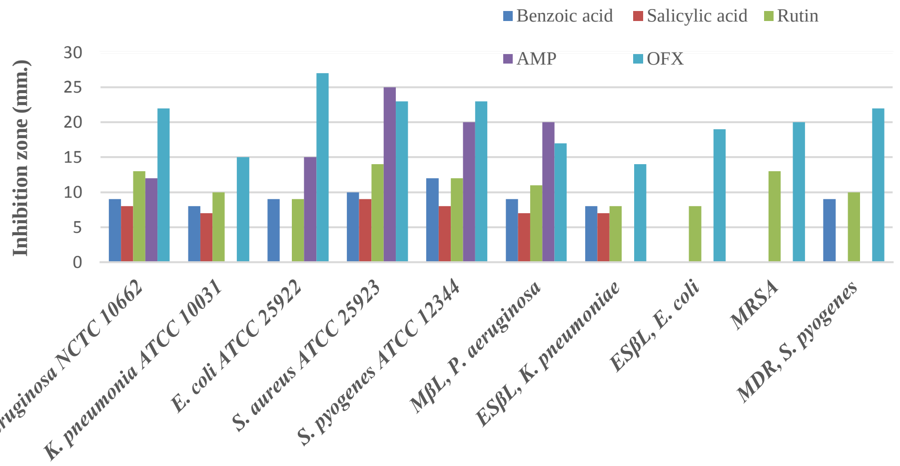


Figure 5. The figure presents HPLC profile of phenolics in *O. marjorana.*

Table 4. The figure presents the conc. of identified free phenols (mg/100 gm DW) within *C. longa* and *O. marjorana* using HPLC.

phenolic compound	*C. longa*	*O. marjorana*
Gallic acid	ND	3.22
Catechol	1.39	ND
p- Hydroxy benzoic acid	1.87	21.3
Caffeine	ND	33.2
Vanillic acid	1.03	ND
Caffeic acid	0.45	2.98
Syringic acid	ND	ND
Vanillin	8.48	53.80
p- Coumaric acid	1.84	11.10
Ferulic acid	2.85	9.20
Rutin	41.62	155.00
Ellagic acid	12.95	18.98
Benzoic acid	70.83	706.12
o- Coumaric acid	0.86	ND
Salicylic acid	2.39	216.00
Cinnamic acid	ND	5.72

In Fiure 6 and Table 5, rutin showed antibacterial activity against all tested bacteria, it produced greatest inhibition zone against *S. aureus* ATCC 25923 (14 mm) with MIC 0.78 mg/mL. Benzoic acid and Salicylic acid showed good patterns of inhibition against some tested bacteria although the MIC values were higher than those for rutin. The MIC values ranged from 3.13 to 25 mg/mL for benzoic acid and from 25 to 100 mg/mL for salicylic acid. The strongest activity of benzoic acid was against *S. aureus* ATCC 25923 and *S. pyogenes* ATCC 12344 (MIC 3.13 and 6.25 mg/mL, respectively), while the strongest activity of salicylic acid was against both *K. pneumonia* ATCC 10031 and MβL, *P. aeruginosa* (MIC 25 mg/mL). Most of the MBC values were slightly higher than the corresponding MIC values while few showing exactly identical values for both assays.



Firure 6. The figure presents antimicrobial assay of benzoic acid, salicylic acid and rutin against selected bacteria.

Table 5. The table presents MIC and MBC (mg/mL) of selected phenols against different bacterial strains.

Bacterial strains	Selected phenolic compounds / (MIC)/(MBC) (mg/mL)		
	Benzoic acid	Salicylic acid	Rutin
P. aeruginosa NCTC 10662	25 / 50	50 / 100	6.25 / 12.5
K. pneumonia ATCC 10031	12.5 / 12.5	25 / 50	6.25 / 6.25
E. coli ATCC 25922	12.5 / 50	-	6.25 / 12.5
S. aureus ATCC 25923	3.13 / 6.25	100 / 100	0.78 / 1.56
S. pyogenes ATCC 12344	6.25 / 6.25	50 / 50	1.56 / 1.56
MβL, *P. aeruginosa*	25 / 50	25 / 50	12.5 / 12.5
ESβL, *K. pneumoniae*	25 / 100	50 / 100	6.25 / 6.25
ESβL, *E. coli*	-	-	12.5 / 12.5
MRSA	-	-	3.13 / 6.25
MDR, *S. pyogenes*	12.5 / 25	-	3.13 / 6.25

- : No inhibition zone; ATCC: American Type Culture Collection; NCTC: National Collection of Type Cultures; ESβL *K. pneumonia*: Extended Spectrun β Lactamase producing *K. pneumonia*; ESβL *E. coli*: Extended Spectrun β Lactamase producing *E. coli*; MβL *P. aeruginosa*: Metallo β lactamase producing *P. aeruginosa*; MRSA: Methicillin-resistant *S. aureus*; MDR: multi-drug resistant.

The results of antitumor assay of benzoic acid, salicylic acid and rutin in different concentrations (graphically represented in Fig 7) expressed a moderate antitumor activity of Rutin and Benzoic acid (IC_{50} 22.7 and 47.8 µg/mL, respectively), while salicylic acid showed weak antitumor activity (IC_{50} 62 µg/mL).

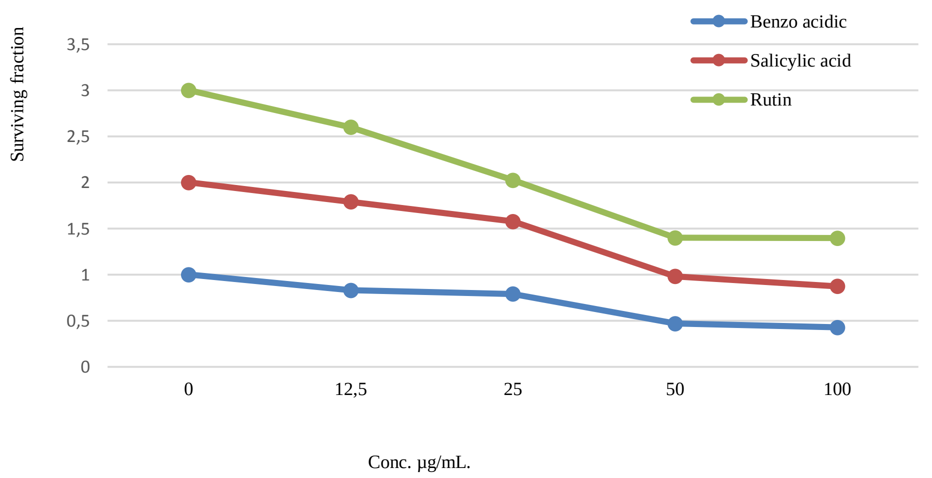


Figure 7. The figure presents potential antitumor assay of selected phenols at different conc. using CACO cell line.

4. DISCUSSION

Bacterial diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antibacterial agents for treatment of bacterial infection is important. Medicinal plants have a long therapeutic history and are still considered to be promising source of medicine in the traditional health care system and act as a source of natural products useful in the development of novel drugs [1].

Extraction is the main step for recovering and isolating phytochemicals from plants and its efficiency is affected by the extraction method and the solvent used, moreover, the percentage yields of the crude extract depend on the polarity of solvents, pH, temperature and extraction time [17]. Water, ethanol and acetone are commonly used for the extraction and the properties of extracting solvents affects the total phenolic content and antioxidant capacity [18]. the results of this study show that, the extraction yield increases with polarity increasing of the used solvent, this may be the reason why yields of ethanol, and acetone extracts are higher than yields of water. These results are in agreement with previous studies in which, extraction yields of rice bran and some medicinal plants decreased in the following order: ethanol > acetone > distilled water [19]. Also ethanol is a better solvents for more consistent extraction of antimicrobial substances from plants compared to acetone and water as solvents [20]. Our results are supported by the study of Tomsone et al. (2012) in which, it was reported that the recovery of polyphenols from plant materials is influenced by the solubility of phenolic compounds in the solvent used [18].

Antibiotic resistance is an *in vitro* phenomenon and the mechanisms of resistance fall into one of the five categories which are enzymatic modification of the antibiotic, reduced antibiotic uptake into the bacteria, and increased efflux of antibiotic from the bacteria, production of a new target site and over expression of the drug target. Medicinal plants have been used against resistant bacterial isolates and previous work concluded that, the plant extract has more pharmacological activity due to their multiple mechanism of action [21].

The results of this study indicated that some plant extracts exhibited a good antibacterial activity and some others are limited as judged by their MIC values, these activities may be due to occurrence of different phytochemicals as flavonoids and alkaloids which have been found to possess anti-microbial and anti-oxidants properties in various studies [22]. The results of this study indicated that extracts showed the larger inhibition zone as well as low MIC values against Gram-positive bacteria when compared with the Gram-negative bacteria. One of the most known reasons is the different nature of cell wall among Gram-positive and Gram-negative bacteria however efflux pump system of Gram-negative bacteria may mediate for such difference [23].

In the present study, *P. aeruginosa K. pneumonia, E. coli, S. aureus, S. pyogenes* of clinical origin were found to be multidrug resistant. MRSA, a Gram positive bacteria which can cause mild to severe disease in immune-compromised patients, are resistant against ampicillin which is a beta-lactam antibiotic that has been used to inhibit the enzyme transpeptidase thus blocking the final stage of cell wall synthesis. Also, *S. pyogenes* is resistant against erythromycin which prevents protein synthesis. Our study showed that, the ethanol and acetone extracts of *C. longa* and *O. marjorana* respectively, had the highest efficiency against MRSA and *S. pyogenes* as was observed from MIC and MBC values. The antibacterial activity of *C. longa* and *O. marjorana* against *P. aeruginosa, K. pneumoniae* and *E. coli* was also studied and it was found that these three Gram negative strains were resistant to most selected commercially available antibiotics. The ethanol and acetone extract of *C. longa* was exceptionally effective against *P. aeruginosa* and *E. coli,* these results in agreement with Chakraborty et al. (2014) who reported that acetone extract of *Curcuma longa* was effective against *P. aeruginosa* and *E. coli* [21]. Also only ethanol extract of *O. marjorana* showed antibacterial activity against resistant *E. coli* as compared to other extracts, while the anti-microbial activity of methanol and water extracts of *O. marjorana* against *S. aureus*, *E. coli,* and *P. aeruginosa* was reported by Adam and Ahmed (2014) [24].

This discrepancy between the used plant extracts and the inhibiting degree of the tested strains can be explained by the fact that, the activity depends on the type, composition, and concentration of the plant extract, and the type of target microorganisms. Many other factors could also be involved such as age of the plant used, freshness of plant, physical factors as temperature and light, time of harvesting, drying method, the effect of solvents and the extraction method on the phenolic contents, and seasonal or intraspecific variation of plant extract composition [25]. Moreover, the negative results do not mean that the bioactive constituents are absent or that the plant is inactive, but active compounds may be present in insufficient quantities so that, the doses level would not be enough to exhibit the inhibitory effect. It is also possible that the plant extracts may be active against other bacterial species that were not tested.

The efficient antimicrobial plant extracts showed remarkable anti-oxidant activity, that may be refer to the polyphenolic components which could be responsible for both activities. The results of this study are in agreement with the previous study of Rajesh et al. (2013) who stated the presence of alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids in alcoholic extract of *C. longa*, moreover, phytochemical screening assay of *O. majorana* revealed the presence of tannins, alkaloids, flavonoids, glycoside, saponin, carbohydrate, terpens and phenolic compound [26].

DPPH method is a preferred method in anti-oxidant assay because it is fast, easy and does not require a special reaction. Our results are in agreement with Złotek et al. (2016) who reported that the free radical scavenging potentials of both acetone and ethanol extracts are higher than hot water extracts [27]. In a previous study, acetone was more effective solvent than methanol for phenols extraction, however, the best solvent for phenolic extraction from horseradish roots was ethanol[23]. Moreover, the maximum polyphenols extraction was obtained in the alcoholic extract of *Bauhinia vahlii* followed by acetone, hot water and chloroform extracts [28].

The results that have been obtained from antitumor activity showed strong activity according to plant screening program of the American National Cancer Institute US NCI which states that, a crude extract is generally considered to have *in vitro* strong antitumor activity if the IC_{50} value following incubation between 48 and 72 hrs is less than 20 µg/mL [29].
Shukla et al. (2016) reported that *C. longa* showed IC_{50} value of 17.8 µg/mL against HeLa cells (cervical cancer cells) [30]. Waller et al. (2016) observed the antitumor activity of all tested concentrations of *O. majorana* oil that caused 70% to 80% inhibition of mammalian cells [5]. The antitumor activity of tested plants may be due to the presence of phytochemicals content in extracts. Even though there was increase in the cell growth inhibition when concentration of plant extract was increased.

HPLC is the best way for chemical characterization and determination of both composition and concentrations of the secondary metabolites of a sample [31]. Like our results, Vallverdú-Queralt et al. (2015) reported that *P*-hydroxybenzoic acid, caffeic acid, *p*- coumaric acid, and ferulic acid were detected in both *C. longa* and *O. marjorana* [32]. Moreover, in our study, rutin was detected in both *C. longa* and *O. marjorana* while it was detected only in *C. longa* in the study of Vallverdú-Queralt et al. (2015) [32]. Also Vallverdú-Queralt et al. (2015) detected synirgic acid, vanillic acid and galic acid in *C. longa* and *O. marjorana* while in our investigation, galic acid and synirgic acid were absent in *C. longa*, also vanillic acid and synirgic acid were absent in *O. marjorana*. Presence or absence of the phenolic acids and differences among its concentrations in all studies can be attributed to genotypic and environmental variation within species and choice of tested plant parts [32].

Nature has always served as an immense source for human that the phenolic compounds which have various properties. This part of the current work indicated various properties of benzoic acid, salicylic acid and rutin that act as antimicrobial and antitumor agents. Benzoic acid, one of the phenolic acids, have been found to inhibit the development of some bacteria [33]. Our results showed that benzoic acid was more active against tested Gram-positive bacteria than Gram-negative bacteria and the same results have been reported by Willey et al. (2008) and Adeshina and Onaolapo (2012) [34,35]. Borawska et al. (2008) reported that, benzoic acid showed antitumor effect on growth of human fibroblasts at the concentration of 0.02% and cell viability at this concentration was below 90% [36]. Also the results obtained by Schroder et al. (2014) stated that benzoic acid have a significant cytotoxicity potential from 24 to 48 hrs using the brine shrimps bioassay [37].

Also our results showed anti-bacterial activity of salicylic acid against most tested bacteria. Gershon and Parmegiani (1962) and El-mougy (2002) reported that Salicylic acid caused an inhibition of all tested bacteria and the zone of inhibition increased as the concentration of salicylic acid is increased [38,39]. Moreover, salicylic acid is a known anticancer effects [40]. Our results are in agreement with those previous studies that showed the antitumor effect of salicylic acid against CACO cell line with IC_{50} 62.0 µg/mL. These results are supported by the study of Vejselova and Kutlu (2015) in which the antitumor effect of salicylic acid was evaluated against A549 cell line and a significant decrease in cell viability was recorded, the IC_{50} value was (6.0 mM) [41]. Spitz et al. (2009) reported that salicylic acid decreased glucose consumption by breast cancer cells MCF-7 and increased caspase activity in CACO cells when applied at such low concentration as 10 µM [42,43].

Many plants, rich in flavonoids, are known possess antimicrobial and antitumor potential [44,45]. Orhana et al. (2010) stated that rutin is a flavonoid that showed antimicrobial activity against both standard Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) at MIC values around 16 mg/mL and standard Gram-positive bacteria (*S. aureus, E. faecalis, and B. subtilis*) at MIC values around 4 - 8 mg/mL [46]. These results are in agreement with our results that rutin showed antibacterial activity against all tested bacteria with MIC ranged between 1.56 to 12.5 mg/mL. Also the present study showed that rutin has a significant antitumor effect against CACO cell line with IC_{50} value 22.7 µg/mL. The results present in the study by Araujo et al. (2013) corroborate the literature demonstrating the cytotoxic effect of rutin against the HL-60 cell line causing loss of cells at the effective doses, and the IC_{50} value was around 14.0 mg/mL [47]. In the study of Dubey et al. (2013), MTT assay demonstrated significant cytotoxic potential of rutin on isolated leucocytes [45].

5. CONCLUSION

The present investigation provided supportive data for the possible use of *Curcuma longa* and *Origanum marjorana* in treatment of various diseases and reveal the possibility of exploring of locally cultivated, new, safe and inexpensive drugs of plant origin that exhibit antibacterial, antioxidant, antitumor properties.

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