In vitro antibacterial, anticancer and antioxidant properties of some oil plant extract

Abeer A. Abu-Zaid [1,2], Majeeda A. Alopidi[2] EL-Sehrawy M.H.[2,3*]

[1]Food Technology Research Inst., Agric Rese. Cent., Giza, Egypt
[2]Biology Dept., Fac. of Science, Taif University, Saudi Arabia
[3]Botany and Microbiology Department, Faculty of Science(Boys), AL-Azher University, Cairo, Egypt
moataz50@yahoo.com

Abstract: This study aimed to evaluate the protective effect of *Rosmarinus officinalis* and *Laurus nobilis* EO as well as *Brassica sp* mustard oil including: antimicrobial, minimum inhibition concentration MIC, antioxidant, and anticancer activity. Average of Essential Oil (EO) extracted from dried *Rosmary* and *Laura* leaves by steam distillation were 0.7-1.5% and 0.8 -1.4% v/w respectively. Meanwhile, the seed of mustard extracted by mechanical press was 39-48 % oil. The chemical constituents of *Rosmary* and *Laura* EO were identified by GC/MS, α- pinen (27.21, 10.56%), 1,8 cineole (18.55, 40.78%) were found to be main components. While mustard oil had Sixteen phenolic compounds identified by HPLC. Physico- chemical properties of oil plants were determined. In conclusion, *Rosmary* and *Laura* EO had a substantial inhibitory effect on all assayed bacteria strains. Meanwhile, Mustard oil was possessed no antibacterial activity against all tested bacteria. The bacterial strains tested were found to be sensitive to Essential Oils studied and showed remarkable antibacterial activity with minimum inhibitory concentrations (MIC) ranging from 25-125 µl/ml. In addition the highest free radical scavenging was recorded 85.53 % at 30µl/ml of *Rosmary* EO. The inhibitory trend in both *Laurus* EO and Mustard oil against HCT and HEPG-2 cell lines was exponential trend whereas in case of *Rosmary* EO the trend was logarithmic. In conclusion, the extracted EO of *Rosmary* and *Laura* as well as Mustarda oil in this study are strongly recommended to use as generally recognized as safe (GRAS) oil.

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1. Introduction

Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices have been used for thousands of years to enhance the flavor, color and aroma of food, also known for their preservative and medicinal value (DeSouza *et al.,* 2005). Some authors showed the antimicrobial activity of several spices and Essential Oils (Fu *et al*., 2007).Volatile compounds of Essential Oil which are the secondary metabolites of plant may act as phytoprorective, they have known as antimicrobial, antifungal (Oka *et al.,* 2000). Phenolic components, also present in Essential Oils (EO), they have been known to possess antimicrobial activity. Some Essential Oils are classified as generally recognized as safe (GRAS) substances, and therefore could be used to prevent post-harvest growth of native and contaminant bacteria (Singh, *et al.*, 2001). Food and Drug administration USFDA, (2006) have been approved EO for food and beverage consumption.

Free radicals and other relative spices cause the oxidation of biomolecules (e.g., protein, amino acid, lipid and DNA) which leads to cell injury and death (McCord, 2000). Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Since ancient times, spices and EO added to different types of food to improve flavors were well known for their antioxidant capacities (Tsai *et al.,* 2005). Antioxidant effect of aromatic plants is due to the presence of phenolic compound (Shahidi and Wanasunda, 1992).

The side effects of some synthetic antioxidant used in food processing have already been documented. These substances can exhibit carcinogenic effects in living organisms (Baardseth, 1989). For this reason, numerous reports have described antioxidants activity present in herbs, and Essential Oil (Hou *et al.,* 2005). Rates, (2001) estimated that around 60% of antitumor and antiinfectious drugs, already on the market or under clinical trial, are made from natural origin.

Rosmarinus officinalis, a member of the family (Lamiaceae) is a flowering plant which grows wild in most Mediterranean countries (Hèthelvi *et al.*, 1987). The Essential Oil of *R. officinalis*, known as the Rosemary oil, is obtained by steam distillation of the fresh leaves and twigs, and the yields range from 0.5 to 1.0% (Tewari and Virmani, 1987). Fourty to fourty nine components were concluded by Derwich *et al*.(2011). They were extracted EO constitute by 65.61% of the total oil and the yields were 0.54% as a

volatile oil. The chemical composition of Rosemary EO, α-pinene(18.25%) was reported as the major component, followed by 1,8 – cineole, camphene, β-myrcene, and borneole. On the other hand, Jiang *et al.* (2011) described major constituents of the *R. officinalis* EO as 1,8-cineol (27.23%) all these compounds amounted to 93.4% (w/w) of EO. Moreover, extract of *Rosmarinus Officinalis L* leaves contains flavonoids, phenols, volatile oil, rosmarinic acid and terpenoids (Almela *et al.,* 2006).

Rosemary oil is confirmed as antimicrobial agent against both Gram-positive and Gram-negative bacteria. They concluded that antimicrobial impacts of the Essential Oil *Rosmery* plant related to the high percentage of α- pinen, camphor, verbenone and 1,8-cineole (Moghtader & Afzal, 2009). Meanwhile, Jiang *et al.* (2011) showed pronounced *Rosmary* oil as antibacterial and antifungal activity than 1,8-cineole and α-pinen against all tested bacteria. Derwich *et al.* (2011) were inhibited bacterial strains at (MIC) values of 48.2µg/mL by *Rosmarinus officinalis* EO. *Rosemary* herbs have been widely used in the traditional medicine, antioxidant, antitumogenic (Lam *et al.,* 2004). Tavassoli and Djomeh, (2011) were reported that highest antioxidant effect of *Rosmary* oil was due to the presence of total phenols (225.08 mg /L and 4.99± 0.054 g/ 100g as gallic acid equivalent) respectively. In contrast, Imelouane *et al.*(2010) indicated that the effect of *Rosmar*y EO was related to nonphenolic components present in oil. *Rosmary* have a potential therapeutic for prevention and treatment of cancer (Parmar *et al.,* 2011). *Rosmary* extract and their components show inhibitory effects on the growth of breast, liver prostate, lung and leukemia cancer cells (Celiktas *et al.*, 2007). It also, repressed the initiation and promotion of tumorigenesis of melanoma and glioma in animal models (Hung *et al.,* 2009).

Laurel (*Laurus nobilis*) is an evergreen tree cultivated in many warm regions of the world, and widely cultivated mainly in Europe and the USA as an ornamental plant (Barla *et al*., 2007). *Laurus nobilis* is a member of the family named Apollo's *Laurel* in mythology. In the study of Ozcan *et al.* (2010) were identified 25 compounds from *Laura* EO, 1.8-Cineol (44.72%), a-Terpinyl acetate (12.95%), Sabinene (12.82%) were the main components. Fang *et al.* (2005) identified six compounds from *L. nobilis* EO which are 10-epigazaniolide, Gazaniolide, Spirafolide, Costunolide, Reynosin and Santamarine. Ten sesquiterpenes, together with 12 known compounds were isolated from leaves of *Laurus nobilis L.* Based on spectroscopic analyses (Julianti *et al.,* 2012). Essential Oils have been shown to possess antibacterial and antioxidant properties (Burt, 2004). The EO of *L. nobilis* has been found to be active against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, and *Shigella flexnerii* which are pathogens of the intestinal tract (Marzouki *et al.,* 2009). *Staphylococcus intermedius* strains were the most susceptible to *Laura* EO, which is scored highest inhibition by *MIC* = 640 µg/ml (Ivanovic *et al*., 2010). *Laura* EO are rich source of biological active compounds which used in treatment cancer and food preservation (Sylvestre *et al*., 2006). Wild laurel has a high level of antioxidant activity (IC_{50} = 1 microg/ml) (Karaalp *et al.*, 2011). *Laura* EO contained active compounds which exhibited moderate to significant cytotoxicity towards K562 leukemia cells (Julianti *et al.*, 2012). The ethanol crude extracts from *L. nobilis* also showed antiproliferative activity to breast cancer cell line (MCF7) with IC50 values 24.49µg/ml (Al-Kalaldeh *et al*., 2010).

The family Brassicaceae has approximately 350 genera, divided into 13 tribes, and has about 3200 species (Joly, 1998). The seed crops of Brassica grown for industrial purposes are rapeseed (*Brassica campestris L.* and *B. napus L.*) and mustard (*B. juncea L.* and *B. carinata A. Br.*), which are usually grown in the arid and semi-arid regions of the world, particularly in Pakistan. Rapeseed is a rich source of oil and protein. The seed contains high percentage of oil with 40–42%. While the seed meal has protein content of 43.6% and has a complete component of amino acids including lysine, methionine and cystine. *Brassica* genus has been intensively studied due to its health benefits. Several studies have been conducted with this *Brassica,* showing antimicrobial activity against Gram-positive bacteria and strong antioxidant activity (Hurtado *et al.,* 2012). Other studies also have shown a relationship between the compounds present in the genus *Brassica* and the prevention of cancer (Ghawi *et al.,*2013). In fact, there is a correlation between health and the volatiles that contribute to the flavour and influence of the organoleptic properties of plant foods (Maarse, 1991). AiLee *et al.* (2010) used EO of mustard to reduce the final cell of *E.coli* CC 25922 than control in Sausage Chicken. Moreover, Kanemaru and Miyamoto (1990) found that mustard extract increased the lag phase of several bacteria including *E.coli, Staphylococcus aureus, Proteus vulgaris, Pseudomonas fragi, Pseudomonas aeruginosa.* Sousa *et al,* (2008) stated that EO of *Brassica hirta* can inhibite *S. aureus* at MIC value of 500 ppm. Some have been shown to be beneficial (like chemoprotective effect against certain cancers in humans) (Padilla *et al.*, 2007). Jacob *et al.*(2011) were reported that *Brassica* family rich in antioxidant such as phenolic, flavonoid. Gonc-alves *et al.* (2012) suggested that the antigenotoxic properties of this extract may be of great pharmacological importance and antioxidant capacities. Ghawi *et al.* (2013) stated

that the addition of powdered mustard seeds to processed food significantly increased the formation of anti cancer component. Therefore, the objectives of the present study are to Extracte oil and Identificate the active compounds present in *Rosmary* as well as *Laura* and *Brassica sp.* (seed oil). Another objective to determine some chemicals and physical properties present in both Essential Oils and *Brassica sp*. (seed oil), then study the protective effect of both Essential Oils and *Brassica sp.* (seed oil) including; antitumor, antioxidant, and antimicrobial activity, also minimum inhibition concentrations, against pathogenic bacteria.

2. Material and Method

2.1. Plant material

Fresh plants of commercial dried leaves *Laura, Rosemary,* and *Mustard seed* were collected from Kingdoom of Saudia Arabia (Taif city). The leaves were dried in a dark place at room temperature (25^0C) for 4 days.The average of moisture content for the dry plant materials was 11%. The dried sample were ground and kept in glass jars till analysis.

2.1. 1.Extraction of EO

Essential oils were isolated by steam distillation from the *Rosemary* as well as *Laurus* powdered leaves for 3 hours as described by Atti –Santos *et al*.(2005). The EO were dried over anhydrous sodium sulfate and stored in glass vials with sealed caps at -18±0.5^0C, till analysis. While mustard seed were expressed by mechanical at room temperature then kept in glass vials sealed caps at-18±0.5^0C, till analysis.

2.1.2. Identification of EO by GC / MS

Gas Chromatographic Mass Spectrum (GC/MS) analysis was carried out according to method described by Atti – Santos *et al.* (2005).

2.1.3. Determination of phenolic compounds

phenolic compounds in mustard oil were determined by HPLC according to the method of Goupy *et al*. (1999).

2.2. Physico – chemical properties of EO

2.2. 1. Peroxide value

Active peroxide content in one gram of products was done by oxidizing potassium iodide to release iodine under the conditions of the method described. Weigh 1g of the oil in a microwave tube you put in an Erlenmeyer flask, add 10 ml of chloroform and shake. Add 15 ml of acetic acid CHCOOH, then 1 ml of saturated aqueous KI, stopper immediately, shake the bottle and leave for 5 min in the dark. Titrate carefully in the presence of starch, iodine released with $Na_2S_2O_3$ solution (0.01 N) until complete discoloration of the solution. The calculation of Pv was given by the formula:

PV = 8000 V / m.

Where: m: is the mass of the test.

V is the volume of N/100 thiosulphate solution.

2.2.2. Evaporation residue

The evaporation residues of EO were determined by the method described in European Pharmacopeia. 2.8.9 (2001).

2.2.3. Specific gravity

The specific gravity of Essential Oils was determined using a pycnometer at 20°C, according to the method reported in British Pharmacopeia (Ph. Eur. methods 2.2.5)(2004).

2.2.4. Refractive index

Refractive index of EO was measured using an Abbe refractometer at 20°C, according to the method described in British Pharmacopeia (Ph. Eur. methods 2.2.6)(2004).

2.2.5. Acid value

The value of EO was determined by the method described in British Pharmacopeia (Ph. Eur. methods 2.5.1)(2004).

2.3. Antioxidant activity DPPH assay

The free radical –scavenging activity" DPPH assay " of,*Rosmary and Laura* EO as well as mustard oil was detected according to the method described by choi *et al*.(2000). Reading was calculated according to the following formula of Yen and Duh (1994).

$$\mathbf{Ip = [(A_B - A_A)/A_B]X\ 100}$$

Where: Ip: Inhibition percentage; A_B: Absorbance values of the blank samples checked after 70 min; A_A: Absorbance values of EO checked after 70 min.

2.4. Antimicrobial activity of EO

The Antimicrobial activity of extracted EO of *Laurus, Rosemary* and Mustard oil were determined by using the disc diffusion method according to Gachkar *et al*.(2007).

2.4. 1. Minimum Inhibitory concentration of Essential Oils

The selected EO were screened against twenty bacterial isolates (M1 to M20). The minimal inhibitory concentration (MIC) was determined only with microorganism that displayed inhibitory zones. MIC was defined as the lowest concentration that inhibited the visible bacterial growth (NCCLS, 2005).

3.5. Evaluation of Cytotoxic Effects of oils

Various concentrations of *Rosmary* and *Laura* Essential Oils as well as Mustard oil sample (50, 25, 12.5, 6.25,3.125 and 1.56 µg) were added and the incubation was continued for 48 h and viable cells yield (using the colon carcinoma cells (HCT) and Hepatocellular carcinoma cells (HEPG-2)) were determined by a colorimetric method. This method was described according to Vijayan *et al*.(2004).

3. Results and Discussion

3.1. Essential oils yield

Average of EO extracted from dried *Rosmary* and *Laura* leaves (commercial) by steam distillation was 0.7-1.5% v/w. This data was in the range of those reported by Ismail, (2012) and Kaya *et al*.(2012). The seed of mustard (commercial) extracted by

mechanical press yield 39-48 % oil. The same finding has been previously demonstrated by Hurtado *et al.* (2012). These results were presented in table (1).

3.1.1. Fractionation and Identification of oils

Results were obtained by GC/MS analysis of the EO of *Rosmary* as well as *Laura,* while mustard oil analyzed by HPLC. These results were shown in Table (1).The extracted *Rosmary* EO had fourty-one compounds, representing 97.94 % of total oils. The major identified compounds of *Rosmary* were α-pinen (27.21%),1,8 cineole (18.55%), camphor (12.69%) respectively. These results are is in agreement with those mentioned in previous studies by Ismail, (2012).On the other hand, Hussain *et al.* (2010) were identified 1,8 cineole and camphor as a major components of *Rosmary* EO.

Essentail Oil of *Laura* had twenty four compounds representing 99.5% of total oils. The major extracted components were 1,8 cineole (40.78%), α-Pinene (10.56%),Sabinene (9.34%). A similar chemical composition of the oil extracted from bay leaves was observed by several authors Ozek (2012). The HPLC analysis showed the sixteen phenolic compounds which extracted from Mustard oil (stable oil). The major components were pyrogaloll (701.63), benzoic (344.60) µg /100g. There are no previous data available in the literature on the quantitative (g/100 gm) and chemical composition analysis of Mustard oil components with which these present results could be compared.The differences in chemical compositions of the *Rosmary* EO could be attributed to climatic effects on the plant, plant part, species, extraction methods and geographical regions (Toncer *et al.*, 2009). Meanwhile, the differences in the EO of *Laura* composition were dependent more on the part of plant and not on sampling season (Marzouki *et al.,* 2008).

3.1.2. Physco- Chemical properties

Results of the physco – chemical properties of *Rosmary* and *Laura* EO as well as Mustard oil are presented in Table 2. All oils in the present study showed a good physco properties. It should be mentioned that, specific gravity is one of the most important criterion, which specify both quality, and purity of any EO. Moreover, the low content of evaporation index (which reflects the constituent of EO that don't evaporate at 100 ^{o}C) indicate high amounts of monoterpenes and vice versa where high content of sequiterpenes increase evaporation residue (5%). A similar study was conducted by Atti-Santo *et al,* (2005). The acid value recorded 2.2 and 3.2mg KOH/g EO *Rosmary, Laura*. Chaouche *et al.* (2011) determined the value of the acid of *Laura* EO with (2244), that was similar to that given by Agnihotri *et al*. (2003). The peroxide value determined in this work only for mustard oil (0.9), and This value was within the range given by Chaouche *et al.*(2011) for *Laura* EO.

3.2. Antimicrobial activity

The inhibition zones measured in millimeter by disc diffusion (Gachkar *et al.*, 2007) method for preliminary antibacterial evaluation of the Essential Oils are presented in Table 3. The *Rosmary* EO was more effective against *Escherichia. coli* M17,18 and *Staphylococcus aureus* M13 at different concentration (15, 16, and 14 mm), while it showed lowest antibacterial activity on *Bacillus subtilis* M05 (5 mm). These results are not agreement with finding by Kim *et al.* (1995) who mentioned that the *Escherichia coli* (Gram-negative) was more sensitive than *Bacillus circulance, Bacillus subtilis* and *Staphylococcus. aureus* (Gram-positive) against all Essential Oils. Furthermore, Bitki, (2011) stated that the *Rosmary* EO did not show any activity on the tested bacteria. Under equal conditions, the differences in the diameter of zones of inhibition can be attributed to the techniques employed (Viljoen *et al.,* 2003). The antimicrobial activities of *Rosmary* EO can be attributed to the presence of, camphor, 1,8 –cineole, two monoterpenes with well documented antibacterial potential (Sivropoulou *et al.,* 1997).

Laura EO possessed little antibacterial activity on *Bacillus subtilis* M02, M06 (3 mm). Maximum activity of *Laura* EO was observed against *Escherichia. coli* M18,20 (8 mm). These results are in agreement with (Derwich *et al.,* 2009). On the other hand, Bitki, (2011) stated that *Staphylococcus. aureus* was sensitive to *Laura* EO. Antimicrobial activity was expressed as minimum inhibitory concentration (MIC). The results of the MIC are listed in table 4. In general, MIC values confirmed the results obtained with the disc diffusion. The Essential Oils had substantial inhibitory effect on all tested bacteria strains. On the other hand, *Laura* EO possessed little or no antibacterial activity on the tested bacteria while *Rosmary* did not show any activity (Bitiki, 2011). *Laura* EO was the most effective against *Escherichia coli strain* M18, *Bacillus subtilis* M07, M10 and *B. circulans* M01, M08 with low MIC value of 25 µl/ml. In contrast, the highest MIC value of Essential Oil of *Laura* was determined as 125 µl/ml for *Escherichia coli strain* M19 and *Bacillus subtilis* M02, M05. These results are in agreement with, Ivanovic *et al.* (2010) stated that the EO of *Laura* was exhibited antibacterial activity against *Staphylococcus. aureus.* While they determined MIC value of 0.35 mg/ml and 1280 µl/ml respectively, which were higher than these results. The major components of this oil, 1,8 – cinole, has been known to exhibit antimicrobial activity against the bacterial strains of (*Escherichia coli, Staphylococcus aureus and Bacillus subtilis)* (Sivropoulou *et al.,* 1997).

Table 1. Main components of *Rosmary* and *Laura* EO and Mustard oil.

No.	Component	RT	Percentage (%) µg/100ml		
			*Rosmary**	*Laura***	Mustard****
1	Syringic	257	0.151		50.00
2	1,6- octadien-3 -ol	612	.85	0.86	701.63
3	pyrogallol	622	0.125		119.88
4	Gallic	648	-		
5	1H- Cyclopropa (a) nephthalene	763	0.26		111.71
6	Protocatchuic	784	0.100		19.0
7	Catechein	817	0.11		
8	Caryophyllene	820	1.16	0.58	46.8
9	Catechol	828	0.146		44.09
10	Chlorogenic	852	0.100		
11	Bicyclo(heptan – 2-ol-trimethyl acetate)	928	1.06	2.99	
12	α-Pinene	934	27.21	10.56	
13	Camphene	945	4.32	4.25	
14	Verbenene	947	1.86		104.0
15	Caffeic	951	-		
16	4,5 epoxy -1- isopropyl- 4 methyle cyclohexen	960	.18		
17	3- Octanone	966	.61		50.97
18	Vanillic	970	-		
19	β-Pinene	971	1.67	2.66	
20	Sabinene	972	1.85	9.34	
21	Myrcene	982	.30	0.49	
22	α - Phylandrene	997	0.52	0.28	
23	α - Terpinene	1009	0.27	0.90	
24	ρ- Cymene	1012	.51	1.80	
25	Limonene	1021	2.10	8.7	
26	1,8 Cinole	1024	18.55	40.78	
27	cyclohexen	1031	0.37	0. 67	69.0
28	Caffien	1036	-		
29	γ-Terpinene	1048	0.68	1.70	
30	Borneol	1060	1.14		
31	Continued Terpinolene	1079	0.88	0.44	
32	Continued Linaloo	1089	3.66	1.33	
33	Mycenol	1104	0.87		
34	Cis-verbenol	1124	1.0		
35	Camphore	1127	12.69		115.91
36	Ferulic	1130	-		
37	Terpinene-4-ol	1166	1.43	0.23	
38	α-Terpineol	1174	3.42	2.44	
39	Myrtenol	1181	.47		
40	Verbenone	1183	3.45	-	
41	Citronellol	1208	0.25		171.0
42	Coumaric	1248	-		344.60
43	Benzoic	1267	-	2.22	208.0
44	Ellagic	1288	-		
45	Geraniol	1234	.38		
46	Borenyl acetate	1272	1.01		
47	Naphthalene	1326	0.77	0.54	
48	Neryl acetate	1341	1.14		
49	β - Caryphyllene	1424	0.32	0.67	165.0
50	Cinnamic	1468	-		8.48
51	Chrysin	1826	-		
52	Thymol	2187	-	3.7	
53	Eugenol	2225	-	2.04	

All compounds listed in order of elution. **RT**: retention time obtained by chromatogram; *: percentage of EO isolated from *Rosmary* were 0.7-1.5% v/w; **: percentage of EO isolated from *Laura* were 0.8-1.4 % v/w; ***: percentage of EO isolated from Mustard oil were 39-48 % v/w.

Table 2. Physco- chemical properties of *Rosemary, Laura* Essential Oil and Mustard oil.

Properties	Essential Oil		
	Rosmary	***Laura***	**Mustard oil**
Specific gravity at 25 ^{o}C	0.9177	0.9278	ND
Refractive index (25 ^{o}C)	1.4673	1.470	1.4805
Evaporation index (%)	1.02	2.00	ND
Acid value (mg KOH/1gm oil)	2.2	3.2	ND
Peroxide value (MeqO2/Kg oil)	ND	ND	0.9
Acidity (as oleic acid %)	ND	ND	0.22%
Color (LOvibond Inch 5.25)			12 yellow - 5 red

ND; not determined

Table 3. Antibacterial activity of *Rosmary, Laura* Essential Oil and Mustard oil estimated by disc diffusion method.

Microbial strains	Diameter of inhibition zone (mm) of Essentail oil		
	Rosmary	***Laura***	**Mustard oil**
Bacillus circulans M01	9	6	ND
Bacillus subtilis M02	7	3	ND
Bacillus circulans M03	8	6	ND
Bacillus subtilis M04	6	5	ND
Bacillus subtilis M05	5	4	ND
Bacillus subtilis M06	7	3	ND
Bacillus subtilis M07	6	4	ND
Bacillus circulans M08	10	5	ND
Bacillus circulans M09	8	5	ND
Bacillus subtilis M10	7	4	ND
Bacillus circulans M11	9	6	ND
Staphylococcus aureu M12	7	5	ND
Staphylococcus aureu M13	14	5	ND
Staphylococcus aureus subsp. aureus M14	10	6	ND
Staphylococcus aureus subsp. aureus M15	9	6	ND
Staphylococcus aureus subsp. aureus M16	10	5	ND
Escherichia coli M17	15	7	ND
Escherichia coli strain M18	16	8	ND
Escherichia coli M19	10	7	ND
Escherichia coli M20	10	8	ND

Values represent the mean inhibition zones of three experiments; **ND:** not detected; diameter of disc: 2 mm.

Table 4: Minimum inhibition concentration of *Rosmary* and *Laura* Essential Oil against tested strains.

Microbial strains	***Rosmary***	***Laura***
	µl/ml *	
Bacillus circulans M01	25	25
Bacillus subtilis M02	50	125
Bacillus circulans M03	25	50
Bacillus subtilis M04	75	75
Bacillus subtilis M05	50	125
Bacillus subtilis M06	25	50
Bacillus sp M07	25	25
Bacillus circulans M08	25	25
Bacillus sp M09	50	100
Bacillus subtilis M10	50	25
Bacillus circulans M11	25	50
Staphyloccocus sp M12	50	100
Staphylococcus sp M13	50	100
Staphylococcus aureus subsp. Aureus M14	25	75
Staphylococcus aureus subsp. Aureus M15	25	100
Staphylococcus aureus subsp. Aureus M16	75	75
Escherichia coli M17	25	50
Escherichia coli M18	25	25
Escherichia coli M19	25	125
Escherichia coli M20	25	100

Value represent the mean inhibition zones of three experiments; **µl/ml * :** µl Essentail oil per tween 80

The highest inhibitory effect of *Rosmary* EO was exhibited against all isolates from *Escherichia coli* and *Bacillus circulans* (except, M09), *Bacillus subtilis* M06, M07 and *Staphylococcus aureus* M14,15, which showed the lowest MIC (25 µl/ml). The previous results are in agreement with finding by Imelouane *et al.* (2010) which concluded that the *G+ve* bacteria more resistant than other tested bacteria against *Rosmary* EO. In addition, Hussain *et al.* (2010) reported that the G +ve bacteria were resistant to the *Rosmary* EO. On the other hand, they also mentioned that the G +ve bacteria were sensitive to the *Rosmary* EO. It could be concluded that the most susceptible strain was *Escherichia coli*, while the most effective Essential Oil was *Rosmary* on all tested bacteria. These data are in agreement with those reported by Erdogan *et al.* (2012). The minor components such as Camphene, Terrpinen -4-ol and α-pinen, were critical to the activity and may have a synergistic effect of patenting influence (Gill *et al.,* 2002), which were in agreement with these previous study.

3. 3. Antioxidant activity

The antioxidant activity of *Rosmary* and *Laura* Essential Oils, and of Mustard oil at different concentrations were determined by using the DPPH (1,1-diphenyl-2-picryl hydrazyl) assay test. Butylated hydroxytoluene (BHT) was used as a reference results table 5. The inhibition percentage of different concentrations of all tested oils were increased with increase of oil concentration. The radical scavenging activity of *Rosmary* EO a grees with the literature of Wang *et al.* (2007). Wang *et al.*(2008) reported that, *Rosmary* EO showed greater activity than its components (α- penine, 1,8 – cinole and camphor). In contrast Hussein *et al.* (2010) concluded that the maximum inhibition observed from *Rosmary officinalis* EO was (59.1%) while the main component of the oil showed poor activity (33.6%). This might be due to the synergistic effect of some components present in the EO of *Rosmary officinalis.* The previous data were not in agreement with finding by Ouchikh *et al.* (2011) they reported that *Laura* EO exhibited highest antioxidant activity, at 20.94. On the other hand, Ozcan *et al.* (2010) were in accordance with this results that recorded 64.28 %DPPH inhibition for the concentration of *Laura* oil 30 µl. The results of this study confirm with the previous results which have been showing the antioxidant properties of Essential Oil from *Laura.* Among the identified compounds in the Essential Oil from *laurel,* methyl eugenol may be considered the main components shown the antioxidant activity. The antioxidant activity of eugenol has been reported several times (Politeo *et al.,* 2007) and they were reports that, monoterpene hydrocarbons (sabinene, terpinene) and oxygenated monoterpenes (1.8-cineole, terpinenol, terpinen-4-ol) have shown antioxidant activity of Essential Oil of *Laura. nobilis L.* was due to these compounds (Ruberto and Barata, 2000). Moreover, Ekren *et al.* (2013) concluded that the highest antioxidant activity was found in *Laura* than other tested plants. The highest antioxidant activity was 70.45 %DPPH inhibition for the concentration of mustard oil at 30 µl. These results are similar to results published by Bones and Rossiter (2006). The same finding was reported by Jacob *et al,* (2011), it could be noticed that the mustard oil had a highest amount from allyl isothyocianate (479 mg /100 g Wt). Moreover, An and Choe, (2012) stated that the Carotenoids and polyphenols in mustard oil were more effective than tocopherols and phospholipids in decreasing oxidation of tuna oil, thus this (Mustard) oil acted as antioxidant activity.

Table 5. Antioxidant activity of *Rosmary, Laura* Essential Oil and Mustard oil at different concentrations.

Oil Concentration µl/ml	***Rosmary* (EO)**			***Laura*(EO)**			**Mustard oil**		
	10	**25**	**30**	**10**	**25**	**30**	**10**	**25**	**30**
***DPPH Inhibition(%)**	**73.08 %**	**84.49 %**	**85.53 %**	**34.05 %**	**55.03 %**	**66.40 %**	**33.16 %**	**64.70%**	**70.45 %**
****BHT** 0.2mg/ml (200ppm)	**80.40%**			**80.40%**			**80.40%**		

* Inhibition(%) Determined by DPPH as free radical scavenging activity producer; ** The BHT is synthetic reference

3.4. Anticancer activity

The anticancer activity of different concentration of *Laura* EO were determined by cell viability assay using the colon carcinoma cells (HCT) and Hepatocellular carcinoma cells (HEPG-2). These cell lines were recommended as one of the models of colon and hepatocellular cancer tissue by the National Cancer Institute (NCI)(Vijayan *et al.*,2004). Fig (1) showing the inhibitory activity of *Laura nobilis* EO against HCT and HEPG-2 cell lines and trend line equations. The inhibitory activity of *Laura nobilis* EO against HCT and HEPG-2 cell lines was best described by the following different equations:

HCT = $3.855e^{0.248\ conc}$ (R^2 = 0.996).The coefficient of determination% (R^2 %) =99.6

HEPG-2 = $4.413e^{0.258conc}$ (R^2 = 0.983)).The coefficient of determination% (R^2 %) =98.3

The two cell lines showed nearly similar inhibitory activity when exposed to *Laura nobilis* EO. As respect to $LC_{50,}$ the inhibitory activity against both HCT and HEPG-2 cell lines, the effect of *Laura* EO was nearly similar for both the two cell lines

(IC_{50} for HCT = 0.4 µl/ml, $IC_{50}$for HEPG-2 = 0.5 µl/ml). These were in agreement with regardless of the United States NCI recommendation. They were stated that a plant extract should be considered as active if it inhibits less than 50% at 50 µg/ml concentration (Mantle *et al*., 2000).The cytotoxicty of *Laura* EO exhibited higher inhibitory effect on HCT cell line than other Essentail Oil. In an earlier study, AL- Kalaldeh *et al.*(2010) showed that *Laura. nobilis* had antiproliferative activity to adeno carcinoma of breast cell line (MCF7) with IC_{50} value 24.49 µg/ml. These results are similar to present study. Furthermore, Julianti *et al.*(2012) were found a high amounts from known sesquiterpene lactones in *Laura* Essential Oil, which had a highly cytotoxic against the A2780 ovarian cancer cell line and K562 Leukemia cells respectively.

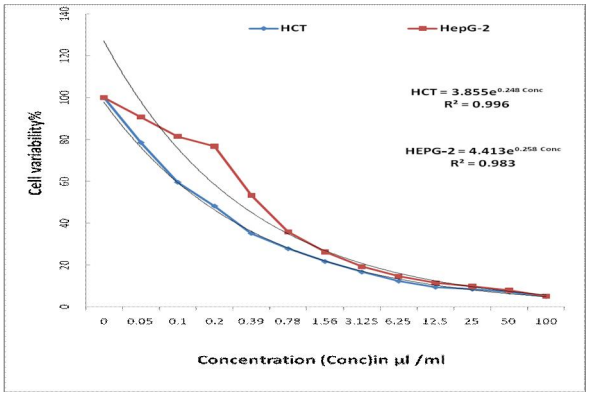

Fig 1. The inhibitory activity of *Laurus nobilis* Essential Oil against

HCT and HEPG-2 cell lines and trend line equations

The inhibitory activity of Mustard oil against HCT and HEPG-2 cell lines and trend line equations were showed in Fig (2). The inhibitory activity of Mustard oil against HCT and HEPG-2 cell lines was best described by the following different equations:

HCT = 3.996 e $^{0.262\ conc}$ (R^2 = 0.981). The coefficient of determination% (R^2 %)=98.1

HEPG-2 = 2.561 $e^{0.269conc}$ (R^2 = 0.987).The coefficient of determination% (R^2%) =98.7

As respect to iC_{50}, the inhibitory activity of Mustard oil was more effective against HEPG-2 cell line (0.1 µl/ml) comparing with HCT line (0.5 µl/ml). These results were in agreement with finding by Thimmulappa *et al.* (2002). They were stated that Mustard seeds were contained plentiful amounts of phytonutrients called glucosinolates. The seeds also contained myrosinase enzymes that can break apart the glucosinolates into other phytonutrients called isothiocyanates. The isothiocyanates in mustard seed (and other *Brassicas*) have been repeatedly studied for their anti-cancer effects. Moreover, isothiocyanates were associated to a protective role against breast, stomach and colon cancer (Taveira *et al.,* 2009). In general, all *B. juncea* genotype had higher of glucosinolate, which were toxic for both humans and animals (Iqbal *et al*., 2008). The addition of Mustard seeds to fully cooked broccoli, ensures to the conversion of. glucoraphanin to anticancer sulforaphane (Ghawi *et al.,* 2013).

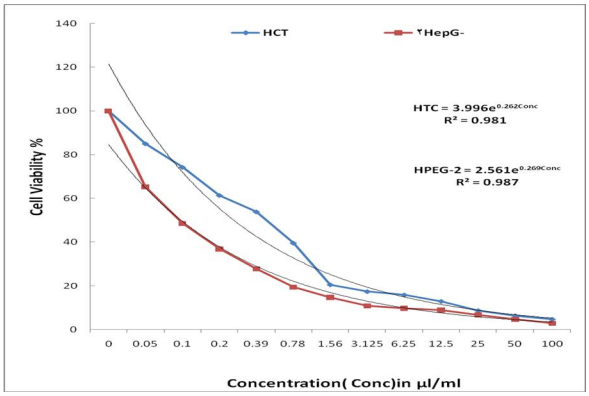

Fig.(2) The inhibitory activity of Mustard oil against HCT and HEPG-2 cell lines and trend line equations.

Fig (3) showing the inhibitory activity of Essential Oil of *Rosmarinus officinalis* against HCT and HEPG-2 cell lines and trend line equations. The inhibitory activity of *Rosmarinus officinalis* EO against HCT and HEPG-2 cell lines was best described by the following equations:

HCT = 21.17 In (x) + 51.40 (R^2= 0.958). The coefficient of determination% (R^2 %) =95.8

HEPG-2 = 25.74 In (x) + 40.64 (R^2= 0.963). The coefficient of determination% (R^2 %=96.3

Concerning the IC_{50}, the inhibitory activity of *Rosmarinus officinalis* EO against HEPG-2 (60.9 µl/ml) showed greater anticancer activity than HCT cell line (94.6 µl/ml). These results were in accordance with Wang *et al.* (2012) they stated that *Rosmary officinalis* Essential Oil exhibited the strongest cytotoxicity towards Human ovarian cancer cell lines (SK-OV-3 and HO-8910) and human hepatocellular liver carcinoma cell line (Bel-7402). Its inhibition concentration 50% (IC_{50}) values on SK-OV-3, HO-8910 and Bel-7402 were 0.025‰, 0.076‰ and 0.13‰ (v/v), respectively. They concluded that the *R. officinalis* EO was showed greater anticancer activity than its components such as (1,8-cineole, α-pinene and β-pinene). It was very difficult to attribute the biological activities of a total EO to one or a few active principles, because an EO always contains a mixture of different chemical

compounds.(Wang *et al*., 2008). Berrington and Lall (2012) stated that the *Rosmarinus officinalis* scored higher toxicity on the Vero cells with an IC_{50} value of 12.03 μg/mL than other tested EO or the results were present in these study. In conclusion, the inhibitory trend in both *Laurus* EO and Mustard oil was exponential trend whereas in case of *Rosmirnus* EO the trend was logarithmic (Figs1-3).

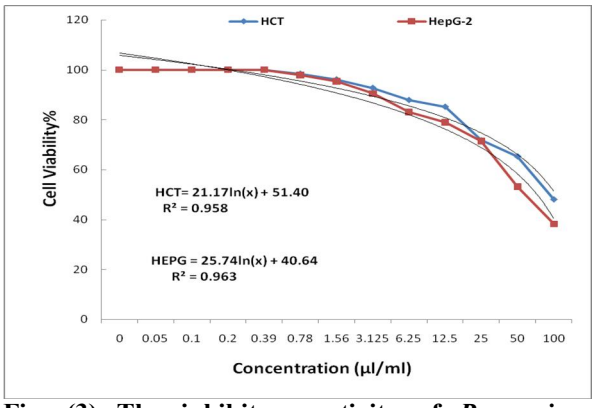

Fig. (3) The inhibitory activity of *Rosmarius officinalis* Essential Oil against HCT and HEPG-2 cell lines and trend line equations.

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