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Full Length Research Paper

Biological effects, antioxidant and anticancer activities of marigold and basil essential oils

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The essential oils isolated from *Tagetes minuta* L. flowers and *Ocimum basilicum* L. herb were analyzed by GC/MS and assessed for antioxidant and *in vitro* and *in vivo* anticancer activities. Also biological effects of these essential oils on normal mice were studied. The major components of marigold essential oil were cis-β-ocimene (54.82%), cis-tagetone (11.50%) and trans-tagetenone (10.78%), cistagetenone (7.10%), dihydrotagetone (6.50%) and limonene (3.82%). The major components of basil essential oil were estragole (75.45%), 1,8-cineole (7.56%), linalool (5.01%), trans-anethole (3.72%) and methyleugenol (3.48%). The DPPH$^{\cdot}$ scavenging activities of both essential oils were determined. 50% effective concentration (EC50) of marigold essential oil (86.35 µg/ml) was higher than basil essential oil (80.84 µg/ml). The anticancer activity of the two essential oils on two human promyelocytic leukemia cell lines (HL-60 and NB4) and experimental animals model cancer cell line (EACC) were investigated *in vitro*. The results indicated that the anticancer activity of marigold essential oil was higher than basil essential oil against NB4 and EACC cell lines, while basil essential oil was higher than marigold essential oil against HL-60 cell line. In *in vivo* study, pre-initiation treatments with the both essential oils were more effective than initiation and post-initiation treatments, respectively on the tumor (EACC) transplanted female mice. Biological effects of both essential oils on normal mice indicated that all the obtained values in all experimental animals were within the normal range.

Key words: *Tagetes minuta*, *Ocimum basilicum*, essential oils, antioxidant, anticancer.

INTRODUCTION

Cancer is the leading cause of death in the world next to cardiovascular diseases. Cancer cells cleverly evade self-demise through apoptosis because of the accumulation of several genetic and epigenetic changes within (Klein, 2004). Agents that can trigger the process of apoptosis in cancer cells are therefore considered potentially important for the development of anticancer chemotherapeutics (Lee, 1999). Of several prescription drugs in use for cancer treatment, almost 75% are derived from plant species (Craig, 1999). It is surprising to note that essential oils, which are found abundantly in nature, have never been exploited for their anticancer potential, although they have found extensive use in perfumery, aromatherapy, food and flavors, etc. since ages (Kumar et al., 2008). Many essential oils or their constituents are known to be the potent antibacterial as well as anti-fungal agents. The application of essential oils in the anticancer therapy may appear unconventional, however, their easy availability, pleasant aroma and low or insignificant toxicity make them more attractive candidates for the long term treatment of various chronic ailments.

The genus *Tagetes* belongs to the Asteraceae family and comprises 56 species, 27 of them annuals and 29 perennials. *Tagetes* sp. are grown all over the world as multi-purpose plants of these species, *T. minuta, T. erecta, T. patula* and *T. tenuifolia,* which are the most common (Vasudevan et al., 1997). *Tagetes* sp. have been used in folk medicine to treat intestinal and stomach diseases and some of them have been found to possess biological activity (Tereschuk et al., 1997; Broussalis et al., 1999). This genus is recognized as a source of very interesting biologically active products, that is, carotenoids used as food colorants and feed additives (Timberlake and Henry, 1986) and possessing anticancer and antiageing effects (Block et al., 1992), essential oils

known for their antibacterial and insecticidal properties (Piccaglia et al., 1996) and flavonoids having pharmacological properties (Tereschuk et al., 1997). Volatile oils of *T. minuta* L. are used as antibacterial (Senatore et al., 2004), flavour components in food products and as perfumes (Chamorro et al., 2008), and have a suppressive biological activity against some insects and pathogens (Vasudevan et al., 1997).

The *Ocimum* genus belonging to the Lamiaceae family (Omer et al., 2008) includes approximately 150 species (Javanmardi et al., 2002), with a great variation in phenotype, oil content, composition, and possibly bioactivity (Simon et al., 1999). *Ocimum sanctum* L. and *Ocimum basilicum* L. are the two basil species that are considered to be promising essential oil crops. The basil essential oil contains pleasant aroma and is known to possess antimicrobial, antioxidant (Bozin et al., 2006; Suppakul et al., 2003) and insecticidal (Aslan et al., 2004) activities. Basil essential oil is a major aromatic agent with applications in various industries, such as the food, pharmaceutical, cosmetic, and aromatherapy industries (Trevisan et al., 2006). There is a great variation of essential oil composition (and aroma) among basil cultivars currently on the international market. The chemical composition of *O. basilicum* essential oils has been intensively investigated throughout the world (Sanda et al., 1998; Yayi et al., 2001), indicating that the estragole chemotype and the linalool -estrgaole one are the most widely distributed (Koba et al., 2009). *O. basilicum* L. named basil is an aromatic herb that has been used traditionally as a medicinal herb in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunctions (Simon et al., 1999). It is also a source of aroma compounds and essential oils containing biologically active constituents that possess antioxidant (Lee *e*t al., 2005), insecticidal (Deshpande and Tipnis, 1997), fungistatic (Reuveni et al., 1984) and antimicrobial properties (Wannissorn et al., 2005).

However, few studies on antioxidant and anticancer activities of marigold and basil essential oils have been performed until now. The objective of the current study was to evaluate antioxidant activity using DPPH radical scavenging assay and anticancer activity against two human promyelocytic leukemia cell lines (HL-60 and NB4) and experimental animals model cancer cells (Ehrlish ascites carcinoma cells, EACC) of *T. minuta* L. (marigold) flowers and *O. basilicum* L. (basil estragole chemotype) herb essential oils. In addition, the present study aimed to investigate the cytotoxicity (if any) induced by these essential oils.

MATERIALS AND METHODS

Plant materials

The fresh flowers of *T. minuta* L. (marigold), family Asteraceae, and herb of *O. basilicum* L. (basil estragole chemotype), family Lamiaceae, were purchased from experimental station of medicinal plants, Faculty of Pharmacy, Cairo University, Egypt. The plant sample was kindly identified by Dr. Mohamed Osama El-Segaee, Professor of Taxonomy, Faculty of Agriculture, Cairo University.

Essential oil isolation

Marigold flower and basil herb samples (100 g) were hydro-distilled in Clevenger-type apparatus (Council of Europe, 1997). The essential oils were collected and dried over anhydrous sodium sulphate. The essential oil samples were stored in the dark at 4°C. The amount of oil obtained from plant material was calculated as:

Oil (% v/w) = observed volume of oil (ml)/weight of sample (g) × 100

GC/MS analysis of essential oil

The essential oils of marigold flower and basil herb were analyzed by GC-MS according to Adams (1989). GC/MS analysis was performed on a Thermoquest-Finnigan Trace GC-MS equipped with a DB-5 (5% phenyl) methylpolysiloxane column (60 m\0.25 mm i.d., film thickness 0.25 µm). The injection temperature was 220°C and the oven temperature was raised from 40°C (3 min hold) to 250°C at a rate of 5°C/min, then held at 250°C for 2 min; transfer line temperature was 250°C. 1 µl of sample was injected and helium was used as the carrier gas at a flow rate of 1.0 ml/min. The mass spectrometer was scanned over the 40 to 500 m/z with an ionizing voltage of 70 eV and identification was based on standard mass library that the National Institute of Standards and Technology (NIST Version 2.0) use to detect the possibilities of essential oil components.

Essential oils antioxidant activity using DPPH radical scavenging assay

The hydrogen atom-or-electron donating ability of the corresponding essential oils was measured from the bleaching of the purple colored methanol solution of DPPH˙. This spectrophotometric assay uses the stable radical, 2,2´-diphenylpicrylhydrazyl (DPPH˙), as a reagent (Brand-Williams et al., 1995). Fifty microliters of various concentrations (25, 50, 75, 100 and 200 µg/ml) of the essential oils in dimethyl sulphoxide (DMSO) were add to 5 ml of a 0.004% methanolic solution of DPPH˙. The reaction mixture was covered and left in the dark at room temperature. After one hour, the absorbance was read against blank at 517 nm. Ascorbic acid was used as standard control. The antiradical scavenging activities of the two essential oils were evaluated in comparison with the reference ascorbic acid as described earlier for extracts. The antioxidant capacity to scavenge the DPPH radical for the oils was calculated by the following equation:

Scavenging effect (%) = $((A_{blank} - A_{sample}) / A_{blank}) \times 100$

Where A_{blank} is the absorbance of control reaction (containing each reagents except the sample), and A_{sample} is the absorbance of sample.

The percentage of Scavenging activity was plotted against the essential oil concentration to obtain the effective concentration (EC_{50}), defined as the essential oil concentration necessary to cause 50% scavenging. Tests were carried out in triplicate.

Essential oils anticancer activity

In vitro studies

Cell growth and viability assay: Three types of cancer cell lines

were used in this study, human promyelocytic leukemia cell lines (HL-60 and NB4) and experimental animals model cancer cells (Ehrlish ascites carcinoma cells, EACC).

Human promyelocytic leukemia cell lines (HL-60 and NB4), obtained from the American Type Culture Collection (ATCC), were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml) in humidified atmosphere containing 5% CO_2 at 37°C for 24 h. Cell counts were determined. HL-60 and NB4 cells were seeded at a density of 3×10^5/ml. After that the cells were treated with different volumes of marigold and basil essential oils to give final concentrations (25, 50, 75, 100 and 200 µg/ml) and incubated under the same condition for another 24 h. The final volume in each experiment was made up to 100 µl with the media containing 1% DMSO. Control cells were treated with the equivalent amount of vehicle dimethyl sulphoxide. After this period, the cell viability was evaluated using trypan blue assay. The viability percentages were calculated according to Bennett et al. (1976). This method depended upon the ability of trypan blue dye to stain the dead cells with blue color. Then for easy counting, hemocytometer slide (under microscope) was used. Each experiment was carried out in triplicate.

Experimental animals model cancer cell line (EACC) was maintained in the National Cancer Institute (NCI) Cairo, Egypt in female Swiss albino mice by weekly intraperitoneal (i.p) transplantation of 2.5×10^6 cells. Similar line was proceeded in our department for the same cells. For *in vivo* and *in vitro* studies, the cells were taken from tumor transplanted animals after ≈ 7 days of transplantation then the number of cells/ml was calculated by using appropriate microscope counting technique (≈$2x10^7$ cells/ml). The cells were centrifuged at 1000 rpm for 5 min, then washed with saline.

The number of cells needed to the test was prepared by suspending the cells in the appropriate volume of saline. The culture medium used was prepared using RPMI 1640 media, 10% fetal bovine serum, 2 mmol/L glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). The viability percentage of tumor cells was measured after incubation with the essential oil as well as saline and DMSO as control. Two ml of medium containing EACC ($2x10^6$ cells) were transferred into a set of tubes, then different volumes of examined essential oil were added into the appropriate tube as well as control to give final concentrations 25, 50, 75, 100 and 200 µg/ml. The tubes were incubated at 37°C under 5% CO_2 for 12 h, then the viability percentage of tumor cells were measured by the method of Bennett et al. (1976) as described before. Each experiment was carried out in triplicate. The percentage of dead cells of each cell lines was plotted against the essential oil concentration to obtain the LC_{50}, defined as the essential oil concentration necessary to cause 50% death.

In vivo studies

Animals: Forty eight healthy female Swiss albino mice weighting 20 – 25 g (7–8 weeks old) were used throughout this experiment. The animals were purchased from the animal house of Helwan Station for Experimental Animals, Helwan, Egypt. They were raised in the animal house of Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt. The animals were housed in polyethylene cages in groups of six mice per cage in a controlled environment condition (25±2°C, 50-60% relative humidity and 12-hour light-dark cycle). All animals were fed standard pellet diet and water *ad libitum* for two weeks (adaption period).

Experimental design

The animals were divided into eight groups and each group contained six mice. Group I served as normal control animals and was given corn oil orally for 16 weeks. Group II animals (tumor control) were transplanted intraperitoneal cavity with EACC at 1×10^6 cells (0.2 ml). Group III mice were treated with EACC (as in Group II) and marigold essential oil (7.5 mg/kg body weight dissolved in corn oil orally). Marigold treatment started after 24 h of EACC transplantation (initiation treatment) and continued daily for the end of the experiment. Animals in Group IV for the post-initiating studies were treated with EACC (as in Group II) and after one week of tumor transplantation (tumor induction period) mice were treated daily with marigold essential oil (7.5 mg/kg body weight dissolved in corn oil orally) till the end of the experiment (post-initiation treatment). Animals in Group V for pre-initiating studies were treated daily with marigold essential oil (7.5 mg/kg body weight dissolved in corn oil orally) for 2 weeks followed by EACC (as in Group II). Marigold treatment continued till the end of the experiment (pre-initiation treatment). Groups VI, VII and VII were treated as in Groups III, IV and V, respectively, but with basil (estragole chemotype) essential oil (10 mg/kg body weight dissolved in corn oil orally) instead of marigold essential oil. The initiation, post-initiation and pre-initiation treatment was used to study the chemopreventive and/or chemotherapeutic efficacies of marigold and basil essential oils in the experimental animals.

Antitumor effect of both essential oils was assessed by observing the changes with respect to EACC, tumor number (all, viable and dead cells) was counted after 12 days of EACC transplantation (Bennett et al., 1976). The mean of survival time (MST) of each group consisting of 6 mice was monitored by recording the mortality daily. The MST of the treated group was compared with that of the tumor control group to calculate the increase in lifespan (ILS) using the following formula according to Rajkapoor et al. (2004):

$$ILS = (T-C)/C \times 100$$

Where T is the MST of treated group and C is the MST of tumor control group.

Lactate dehydrogenase (LDH) activity was determined in the supernatant of tumor cell suspension (EACC) according to Legrand et al. (1992) and Young (2001).

Biological effects of marigold and basil essential oils

Animals

Eighteen male Swiss albino mice weighting 20 – 25 g (7 – 8 weeks old) were used throughout this experiment. Animals were obtained and adapted as in the previous experiment.

The animals were divided into three groups and each group contained six animals. Group A (normal control) was given corn oil orally. Group B (marigold) animals were treated daily with marigold essential oil (7.5 mg/kg body weight dissolved in corn oil orally) and Group C (basil) animals were treated daily with basil essential oil (10 mg/kg body weight dissolved in corn oil orally) for three months to study the cytotoxicity (if any) induced by these essential oils. At the end of the experimental period (3 months), the animals were killed by cervical decapitation. Blood was collected. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min.

Biochemical analyses

Serum glucose was determined according to Trinder (1969), total soluble proteins and albumin were determined according to Hoffman (1966) and Tietz (1995), respectively, but globulin was calculated by the difference between total protein and albumin. Total cholesterol and triglycerides were determined according to Allain et al. (1974) and Fossati and Prencipe (1982), respectively.

Serum uric acid, urea and creatinine contents were determined according to Tietz (1995), First (2003) and Faulkner and King (1976), respectively. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured colourimetricaly in serum according to the method described by Reitman and Frankel (1957). Serum alkaline phosphatase was determined according to the method of Kind and King (1954). Lactate dehydrogenase (LDH) activity in serum was determined according to the method of Young (2001).

Statistical analysis

Statistical analyses (standard deviation ‘‘SD’’ and standard error ‘‘SE’’) was carried out according to Fisher (1970). LSD (Least significant difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969). The statistical package for social science S.P.S.S. (1999) program version was used for all analysis.

RESULTS AND DISCUSSION

Essential oils composition

The hydro-distillation of *T. agetes minuta* flowers (marigold) and the herb of *O. basilicum* (basil) yield 0.66% (v/w) and 0.85% (v/w), respectively. The GC-MS analysis of marigold (flowers) and basil (herb) essential oils are presented in Table 1. It seems that there were no similarities among chemical compositions of the two essential oils.

A total of 17 components constituting 97.71% of marigold (flowers) essential oil were identified and the major components were cis-β-ocimene (54.82%), cis-tagetone (11.50%), trans-tagetenone (10.78%), cis-tagetenone (7.10%), dihydrotagetone (6.50%) and limonene (3.82%). All other components were present in amount lower than 1%. Our results agree with those reported by Babu and Kaul (2007), Chamorro et al. (2008), Lo´pez et al. (2009) and Senatore et al. (2004).

Twenty-three compounds constituting 98.97% of basil (herb) essential oil have been identified. The major components were estragole (75.45%), 1,8-cineole (7.56%), linalool (5.01%), trans-anethole (3.72%) and methyleugenol (3.48%). All other components were represented in amount lower than 1%. These results are similar to that obtained by Koba et al. (2009), Omer et al. (2008) and Yayi et al. (2001) who reported that basil (estragole chemotype) essential oil contained mainly estragole with a little amount of 1,8-cineole and linalool.

In the case of marigold essential oil components, hydrocarbons (cis-β-ocimene) are the main group, with oxygenated compounds as a minor fraction, while oxygenated compounds are the main group in basil essential oil.

Antioxidant activities of essential oils

The DPPH free radical scavenging activities of marigold (flower) and basil (herb) essential oils at various concentrations were determined and compared with that of the standard antioxidant ascorbic acid (Table 2). All the tested samples showed lower DPPH radical scavenging activity when compared with the standard. The highest antioxidant scavenging effect (%) was obtained with ascorbic acid (94.10%) for concentration of 200 µg/ml, while it was 73.40 and 70.83% for 200 µg/ml concentration of marigold and basil essential oil, respectively when recorded after 60 min. No significant different were found between the scavenging effect of both plants for the same concentration. The essential oils of both plants (200 µg/ml) reduced the concentration of DPPH free radical with an efficacy near 75 µg/ml concentration of standard antioxidant. Both plants essential oils were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH reaching 50% of reduction with EC_{50} values as follows: EC_{50} (marigold) = 86.35 µg/ml; EC_{50} (basil) = 80.84 µg/ml and EC_{50} (ascorbic acid) = 41.2 µg/ml. Statistical analysis and the EC_{50} values indicated that the antioxidant activity of the marigold essential oil was as similar as that of the basil essential oil. The quantity of marigold and basil essential oils required were about 2.1 and 1.96 fold, respectively when compared with the standard antioxidant ascorbic acid. The antiradical scavenging activity of oils might be attributed to the replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds as a result of their hydrogen donating ability (Brand-Williams et al., 1995). Cetkovic et al. (2004) showed antioxidant activities of marigold by various antioxidant assays, including DPPH radical assay, hydroxyl radical assay, and peroxyl radical assay. Bashir and Gilani (2008) investigated the antioxidant properties of marigold flowers by DPPH assay. They found that IC_{50} of marigold flowers was 18.04 µg/ml (ethanol crude extract). The essential oil of marigold has shown antioxidant activity (Juliani et al., 2004). Trevisan et al. (2006) investigated the antioxidant properties of *Ocimum* species essential oils by DPPH assay. The author showed strong antioxidant capacity of *O. basilicum* essential oil. Pripdeevech et al. (2010) found that IC_{50} of *O. basilicum* (rich in methyl chavicol) was 98 µg/ml (DPPH assay). Juliani and Simon (2002) evaluate the antioxidant activity of different basil essential oils. In all basils, the essential oil contribution to the total antioxidant activity was low.

Anticancer activities of essential oils

In vitro

In order to understand the effect of marigold and basil essential oils on human promyelocytic leukemia and experimental animal model cancer cells, experiments were conducted using cultured HL-60 and NB4 cell lines and EACC cell line. Results of the viability were

Table 1. Chemical composition of marigold (flowers) and basil (herb) essential oils.

Compound	Peak area (%)	
	Marigold	Basil
α-pinene	0.12	0.09
Myrecene	0.15	0.03
Limonene	3.82	0.24
Sabinene	0.71	0.43
P-Cymene	0.05	-
β-phellandrene	0.08	-
Cis-β-ocimene	54.82	-
1,8-Cineole	-	7.56
Trans-Linalool oxide	0.26	0.36
Linalool	0.07	5.01
Dihydrotagetone	6.50	-
Cis-tagetone	11.50	-
Trans-tagetenone	10.78	-
Cis-tagetenone	7.10	-
Camphor	-	0.28
Borneol	0.12	0.07
Estragole (methyl chavicol)	-	75.45
Trans-anethole	-	3.72
(E)-Isoeugenol	-	0.05
α-Copaene	-	0.06
β-Elemene	-	0.13
Methyleugenol	-	3.48
Piperitone	0.30	-
β-Caryophyllene	0.81	0.05
Trans-α-Bergamotene	-	0.70
β-bisabolene	-	0.10
trans-β-Farnesene	-	0.05
α-Cadinene	-	0.06
α-Humulene	0.52	0.21
Caryophyllene oxide	-	0.74
α-Cadinol	-	0.10
Total identified compounds	**97.71**	**98.97**

measured using trypan blue assay. The results found that the incubation of HL-60 and NB4 cells with marigold and basil essential oils at all concentrations (25 – 200 µg/ml) for 24 h reduced the viability of these cells. The dead cells were increased by increasing the concentration of both plants essential oils (Table 3). The highest HL-60 dead cell (%) was recorded by basil essential oil (82.33%) for concentration of 200 µg/ml, while it was 62.11% for 200 µg/ml concentration of marigold essential oil.

On the contrary, the highest NB4 dead cells (%) was recorded by marigold essential oil (81.87%) for concentration of 200 µg/ml, while it was 73.38% for 200 µg/ml concentration of basil essential oil. It must be noticed that there were no significant difference in dead cells (%) of NB4 cell line between the concentrations (100 and 200 µg/ml) of marigold essential oil (Table 3). Also, no significant different in dead cells (%) of NB4 cell line between marigold at 100 µg/ml concentration and basil at 200 µg/ml concentration. The LC_{50} values were determined from the graphs of the essential oils on HL-60 and NB4 cell lines. Marigold essential oil showed potent cytotoxic effects with the LC_{50} values of 108.8 µg/ml in HL-60 cell line and 66.3 µg/ml in NB4 cell line, whereas basil essential oil gave the LC_{50} values of 78.9 µg/ml in HL-60 cell line and 92.2 µg/ml in NB4 cell line. The LC_{50} values indicated that the anticancer activity of marigold essential oil was higher than basil essential oil against NB4 cell line. On the other hand, the anticancer activity of basil essential oil was higher than marigold essential oil against HL-60 cell line. The results presented in Table 4 pointed out the effects of marigold and basil essential oils

Table 2. The DPPH free radical scavenging activities of marigold and basil essential oils.

Sources	Concentration (µg/ml)	Scavenging effect (%)
Marigold	25	21.13±2.32^{gh}
	50	28.00±3.79^{gh}
	75	39.10±2.98^{ef}
	100	58.50±4.49^{d}
	200	73.40±2.03^{b}
Basil	25	18.73±2.04^{h}
	50	31.00±2.31^{fg}
	75	44.50±2.18^{e}
	100	63.07±4.05^{cd}
	200	70.83±2.62^{bc}
Ascorbic acid	25	25.17±2.69^{gh}
	50	61.77±3.44^{cd}
	75	80.20±3.57^{b}
	100	90.47±4.07^{a}
	200	94.10±4.57^{a}
LSD		9.41

The values are means ± SE. The mean values with different small letters within a column indicate significant differences (P < 0.05).

Table 3. Effect of marigold and basil essential oils on cells viability of HL-60 and NB4 cells after 24 h of treatment.

Sources	Concentration (µg/ml)	HL-60		NB4	
		Dead cells (%)	Viable cells (%)	Dead cells (%)	Viable cells (%)
Control	0	0.00^{g}	100	0.00^{g}	100
Marigold	25	19.40±1.45^{f}	80.60	17.70±1.30^{f}	82.30
	50	26.83±1.30^{e}	73.17	31.50±2.02^{e}	68.50
	75	37.00±2.31^{d}	63.00	59.57±2.60^{c}	40.43
	100	48.50±3.04^{c}	51.50	77.29±2.24^{ab}	22.71
	200	62.11±2.22^{b}	37.89	81.87±4.05^{a}	18.13
Basil	25	11.17±1.68^{g}	88.83	20.10±1.16^{f}	79.90
	50	26.30±1.42^{e}	73.70	28.80±1.01^{e}	71.20
	75	40.74±1.96^{d}	59.26	46.27±2.33^{d}	53.73
	100	53.07±2.31^{c}	46.93	65.08±3.07^{c}	34.92
	200	82.33±5.70^{a}	17.67	73.38±2.22^{b}	26.62
LSD		6.13		6.62	

The values are means ± SE. The mean values with different small letters within a column indicate significant differences (P < 0.05).

on cells viability of EACC. The incubation of EACC with marigold and basil essential oils at all concentrations (25 - 200 µg/ml) for 12 h reduced the viability of these cells. The increase of both plant essential oils concentration increased the percentage of dead cells. The highest EACC dead cells (%) was recorded by marigold essential oil (100%) for concentrations of 100 and 200 µg/ml, while it was 93% for 200 µg/ml concentration of basil essential oil. No significant difference in dead cells (%) of EACC between concentrations 75, 100 and 200 µg/ml of marigold essential oil and concentrations 100 and 200 µg/ml of basil essential oil. In general, it was shown that

Table 4. Effect of marigold and basil essential oils on cells viability of EACC cells after 12 h of treatment.

Sources	Concentration (µg/ml)	Dead cells (%)	Viable cells (%)
Control	0	0.00^{e}	100
	25	6.90±3.11^{e}	93.10
	50	91.36±8.05^{b}	8.64
Marigold	75	98.92±1.88^{ab}	1.08
	100	100.00±0.00^{a}	0.00
	200	100.00±0.00^{a}	0.00
	25	25.88±5.80^{d}	74.12
	50	79.67±2.79^{c}	20.33
Basil	75	84.01±4.6^{c}	15.99
	100	92.50±5.41^{ab}	7.50
	200	93.00±5.29^{ab}	7.00
LSD		7.18	

The values are means ± SE. The mean values with different small letters within a column indicate significant differences (P < 0.05).

the anticancer activity of marigold essential oil was higher than basil essential oil against EACC.

In vivo

The effect of marigold and basil essential oils initiation, post-initiation and pre-initiation treatments on survival of tumor (EACC) transplanted female mice and the number of EACC (all, dead and viable) and also LDH activity in supernatant of tumor cell suspension are shown in Tables 5 and 6. The mean survival days for the untreated tumor control group were 14±0.45 days. The different marigold and basil essential oils treatments of tumor (EACC) transplanted female mice significantly ($p<0.05$) increase MST when compared with tumor control. It was found in the present study that marigold and basil essential oils were more effective during pre-initiation treatment than initiation and post-initiation treatments, respectively. The highest mean survival time (days) was recorded by marigold pre-initiation treatment (48 days) followed by initiation treatment (37.17 days), while it was 34 and 30 days for basil pre-initiation and initiation treatments, respectively. Low effects were observed for post-initiation treatments on MST of tumor transplanted animals. The highest increase in lifespan was observed by pre-initiation marigold group (242.86%).

However, high significant ($p<0.05$) reduction in the tumor cells number (Table 6) of initiation, post-initiation and pre-initiation marigold groups (1.96, 2.04 and 4.64-fold) and initiation and pre-initiation basil groups (1.42 and 2.50-fold) were observed when compared with tumor control. No significant change in tumor cells number was found between post-initiation basil group and tumor control group. Cells number of tumor control animals was 450×10^{6}/ml. The highest reduction was recorded by marigold pre-initiation treatment (97×10^{6} cells/ml) followed by basil pre-initiation treatment (180×10^{6} cells/ml). The reduction of tumor cells number was 1.85 fold decreased in pre-initiation marigold group when compared with basil ones. On the other hand, there was significant ($p<0.05$) increase in dead cells (%) in all treated groups (except post-initiation basil group) when compared with tumor control group. The highest dead cells (%) was recorded by marigold pre-initiation treatment (9.51-fold) followed by basil ones (6.90-fold) when compared with tumor control. These results are in line with that of LDH activity in the supernatant of tumor cell suspension. Cells that have lost membrane integrity release lactate dehydrogenase (LDH) into the surrounding medium. The measurement of the release of LDH from damaged (dead) cells gives an indicator of cytotoxicity (Hernandez et al., 2003). The results indicated that the increase in dead cells increases LDH activity in supernatant of tumor suspension. The highest significant ($p<0.05$) increase in LDH activity was observed by marigold pre-initiation treatment followed by basil ones when compared with tumor control as similar as in dead cells (%). Legrand et al. (1992) found a significant correlation between the number of dead cells, determined by Trypan Blue staining, and LDH activity measurements in the supernatant of hybridoma strains. These results (Tables 5 and 6) indicated that the increase in lifespan was correlated with the decrease in cells number and the increase in dead cells (%). In general, it was shown that the anti-tumor effect of marigold essential oil was higher than basil essential oil. Also, pre-initiation treatments with both essential oils were more effective than initiation and

Table 5. Effect of marigold and basil essential oils treatment on the survival of tumor (EACC) transplanted female mice.

Treatment	Mean survival time(days)	Increase in Lifespan (%)*
Normal control	Morethan120day	More than 757.14
Tumor control	14.00±0.45^{g}	-
Marigold		
Initiation	37.17±0.94^{b}	165.5
Post-initiation	19.50±0.85^{f}	39.29
Pre-initiation	48.00±1.03^{a}	242.86
Basil		
Initiation	30.00±1.12^{d}	114.29
Post-initiation	22.50±0.99^{e}	60.71
Pre-initiation	34.00±0.68^{c}	142.86
LSD	2.57	

The values are means ± SE. The mean values with different small letters within a column indicate significant differences (P < 0.05). * Relative to tumor control.

Table 6. Effect of marigold and basil essential oils on EACC number and lactate dehydrogenase activity in the tumor of transplanted female mice.

Treatment	Number of cells/ml*10^{6}	Dead cells(%)	Viable cells(%)	LDH activity(U/L)
Tumor control	450±25.04^{a}	4.02±0.43^{e}	95.98	12.19±0.87^{d}
Marigold				
Initiation	230±12.71^{c}	18.06±2.53^{c}	81.94	23.40±1.66^{b}
Post-initiation	220±10.56^{c}	13.43±1.11^{d}	86.57	17.50±1.29^{cd}
Pre-initiation	97±4.74^{d}	38.25±1.54^{a}	61.75	30.00±1.54^{a}
Basil				
Initiation	317±17.67^{b}	20.02±1.12^{c}	79.98	17.20±0.68^{c}
Post-initiation	470±24.22^{a}	7.23±0.98^{e}	92.77	17.40±1.39^{c}
Pre-initiation	180±10.64^{c}	27.74±2.04^{b}	72.26	24.00±2.17^{b}
LSD	47.74	3.53		4.19

The values are means ± SE. The mean values with different small letters within a column indicate significant differences (P < 0.05).

post-initiation treatments, respectively on the tumor (EACC) transplanted female mice. This may recommend the use of these essential oils as preventive agents against tumor. These essential oils significantly prevent the development of tumor (decrease total EACC number and increase dead cells).

Pessoa et al. (2006) described the antitumor activity of some species belonging to the family Asteraceae, and showed that the hydroalcoholic extract of *T. minuta* leaves (56 mg/Kg) was effective in inhibiting Walker carcinoma cell growth by 51% in rats. Ickes et al. (2006) revealed that the whole flowering plants of *T. minuta* were significantly active against the Lewis lung carcinoma *in vivo*. Chemo-preventive response of basil leaf extract was evident from the reduced tumor burden (the average number of papillomas/mouse), as well as from the reduced percentage of tumor bearing-animals (Dasgupta et al., 2004). Basil has shown antioxidant, antimicrobial and antitumor activities due to its phenolic acids and aromatic compounds (Gutierrez et al., 2008; Hussain et al., 2008).

Basil (*O. basilicum*) has been reported to be cytotoxic to human cancer cells (Manosroi et al., 2006). Also, Lawrence (1993) reported that *O. sanatcutm* and *O. basilicum* possess antitumor activity in mice. Taie et al. (2010) suggested that basil oil might be good innovative therapeutic strategies against cancer (Ehrlich ascites carcinoma cells).

Biological effects of marigold and basil essential oils

Tables 7 and 8 display the biological effects of marigold and basil essential oils on normal mice. This part of study was conducted to determine the safety of using the marigold (flower) and basil (herb) essential oils as antioxidant and anti-tumor agents. The oral administration of marigold essential oil to mice for 3 months showed no significant changes on serum glucose, albumin, globulin, total cholesterol, creatinine and uric acid; while total protein and urea were significantly ($p<0.05$) increased and triglycerides was significantly decreased when compared with normal control group. In connection, all biochemical parameters were not significantly changed under the effects of basil essential oil administration except glucose level (decreased) and creatinine (increased) when compared with normal control.

Serum ALT, AST, ALP and LDH activities (liver functions) as affected by oral administration with marigold and basil essential oils to mice were determined (Table 8). It is clear that no adverse effects were noticed on serum ALT, AST and ALP activities when administered with marigold and basil essential oils compared with normal control. Also, no change was observed in serum LDH activity of marigold treated animals, but it significantly ($p<0.05$) increased in basil treated animals (52.01 U/L). Although all the obtained values in all experimental animals were within the normal range according to Mitruka and Rawnsley (1979).

The results of marigold group are similar to that obtained by Odeyemi et al. (2008) who reported that *T. minuta* (leaves) essential oil did not produce any significant effect on serum albumin, globulin, total protein, urea and creatinine in Wistar rats The author also found that administration of the essential oil did not produce any significant effect on the serum alanine transaminase but increase the activity of γ-glutamyl transferase. On the other side, experimental studies on albino rats reported that leaf of basil had hypoglycemic effect. While human study also showed significant decrease in fasting and postprandial blood glucose levels during treatment with basil leaves (Agrawal et al., 1996; Rai et al., 1997). These previous experiments may be interpreted the glucose significant decrease in basil group. The serum proteins evaluated in this study are useful parameters to indicate impairment in the functional capacity of the liver and kidney (Naganna, 1989). The fact that there was no alteration in the levels of albumin, globulin, and total protein indicate no toxicity of the essential oils on the liver.

Urea is the nitrogen-containing metabolic product of protein catabolism. The increase in serum urea concentration may be attributed to impairment and indicates renal dysfunction (Yakubu et al., 2003). The change in urea content was not noticeably.

Creatinine is metabolic by-product of muscle metabolism. No alteration in creatinine content in marigold group was found, but the increase in serum creatinine content in basil group indicates glomerular and tubular dysfunction (Chawla, 1999). Therefore, the effect produced by basil essential oil on the indices of kidney damage investigated in this study suggests toxicity.

Serum enzymes measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. Therefore, the increase in serum LDH activity of basil group may indicate tissue damage leading to leakage of tissue enzyme to the serum (Wills, 1985). The result of the present study has shown that the oil of *O. basilicum* is of mild toxicity.

Essential oils and their main components possess a wide spectrum of biological activity, which may be of great importance in several fields, from food chemistry to pharmacology and pharmaceutics (Cristani et al., 2007). The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS), as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food (Kabara, 1991).

Essential oils rich in monoterpenes are recognized as food preservatives (Helander et al., 1998; Ruberto and Baratta, 2000), and monoterpenic essential oils are natural antioxidants (Yanishlieva et al., 1999) that are active against certain cancers (Kris-Etherton et al., 2002). Indeed, a number of dietary monoterpenes have antitumoral activity that can prevent the formation or progress of cancer and cause tumor regression.

Most of the principle components present in marigold and basil essential oils are monoterpenes. Monoterpenes have shown prevention of mammary, lung, skin, liver and forestomach cancers in rat models (Haag et al., 1992). In the present study, the inhibition of human promyelocytic leukemia cells (HL-60 and NB4) and EACC (*in vitro* and *in vivo*) may be due to the presence of monoterpenes. The difference in antioxidant and anticancer effects between the two essential oils is attributable to the chemical composition of each essential oil. Analysis of marigold (flowers) essential oil showed cis-β-ocimene, cis-tagetone, trans-tagetenone, cis-tagetenone, dihydrotagetone and limonene as the major constituents. Also, estragole, 1,8-cineole, linalool, trans-anethole and methyleugenol were the major constituents in basil (herb) essential oil. These compounds are known to possess antioxidant and anticancer properties. The anticancer and antioxidant activities of marigold essential oil may be due to its content of cis-β-ocimene (monoterpene hydrocarbon), cis-tagetone, trans-tagetenone, cis-tagetenone, dihydrotagetone and limonene, which is considered by some researchers to be a potential chemopreventive agent (Crowell, 1999) with value as a dietary anticancer tool in humans (Tsuda et al., 2004). On the other side, the antioxidant and anticancer activities of basil oil may be attributed to the major contents of

Table 7. Serum biochemical parameters of normal male mice administrated with marigold and basil essential oils.

Serum biochemical parameter	Normal control	Marigold	Basil	LSD
Glucose (mg/dl)	75.60±1.94^{a}	76.39±3.83^{a}	66.83±1.74^{b}	8.07
Total protein (g/dl)	5.38±0.18^{b}	6.10±0.87^{a}	5.51±0.24^{b}	0.55
Albumin (g/dl)	3.50±0.13^{a}	3.83±0.13^{a}	3.81±0.08^{a}	0.34
Globulin (g/dl)	1.70±0.18^{ab}	2.26±0.07^{a}	1.88±0.12^{b}	0.39
Triglycerides (mg/dl)	131.57±6.62^{a}	102.90±5.14^{b}	116.96±3.32^{ab}	15.7
Total cholesterol (mg/dl)	66.00±2.44^{a}	72.80±3.50^{a}	69.77±4.02^{a}	4.02
Creatinine (mg/dl)	0.82±0.02^{b}	0.90±0.05^{b}	1.02±0.04^{a}	0.11
Uric acid (mg/dl)	2.74±0.10^{a}	3.01±0.09^{a}	2.90±0.13^{a}	0.33
Urea (mg/dl)	12.80±0.28^{b}	14.32±0.26^{a}	13.43±0.32^{b}	0.87

The values are means ± SE. The mean values with different small letters within a raw indicate significant differences ($P < 0.05$).

Table 8. Serum ALT, AST, ALP and LDH activities of normal male mice administrated with marigold and basil essential oils.

Serum	Normal control	Marigold	Basil	LSD
ALT (IU/L)	12.12±0.44^{a}	13.50±0.57^{a}	13.87±0.76^{a}	1.82
AST (IU/L)	31.40±2.76^{a}	28.84±2.42^{a}	30.45±3.01^{a}	8.26
ALP (IU/L)	13.50±0.99^{a}	15.40±1.40^{a}	14.70±0.88^{a}	3.36
LDH (U/L)	44.01±2.11^{b}	42.59±2.43^{b}	52.01±3.09^{a}	11.19

The values are means ± SE. The mean values with different small letters within a raw indicate significant differences ($P < 0.05$).

estragole (Pripdeevech et al., 2010; Shahat et al., 2011), 1,8-cineole (Meftahizade et al., 2011), linalool, trans-anethole (Shahat et al., 2011) and methyleugenol (Mimica-Dukić et al., 2010). Cha et al. (2010) found that 1,8-cineole induces apoptosis in KB cells (human oral epidermoid carcinoma cells) via mitochondrial stress and caspase activation. Kamatou and Viljoen (2008) reported that linalool and linalool-rich essential oils are known to exhibit various biological activities, such as antimicrobial, anti-inflammatory, anticancer and antioxidant properties.

Conclusion

The present study shows that essential oils of marigold and basil may be potentially used as good sources of antioxidants and anticancer (HL-60, NB4 and EACC). The overall results obtained from marigold essential oil were better than those obtained from basil essential oil. In addition, the marigold essential oil is more effective on NB4 cell line than HL-60 cell line; On the contrary, basil essential oil is more effective on HL-60 cell line than NB4 cell line. On the other hand, marigold essential oil is more effective on EACC (*in vitro* and *in vivo*) than basil essential oil. In the context, essential oils have preventive efficacies against development of tumor in transplanted animals. Marigold and also basil essential oils are possible sources of antioxidant and anticancer compounds. These observations prompt the necessity for further studies, focusing on the isolation and structural elucidation of their antioxidant and anticancer compound/s, since they have potential use as therapeutic agents in managing diseases associated with free radicals, and also employed as additives in the food or cosmetic industries. Finally, the present results clearly refer to the possibility of using marigold and basil or their essential oils as antioxidant and anticancer agents, taking into consideration the mild toxicity of basil essential oil. However, studies need to be carried out on human patients.

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