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Phytochemical composition, antifungal, antiaflatoxigenic, antioxidant, and anticancer activities of *Glycyrrhiza glabra* L. and *Matricaria chamomilla* L. essential oils

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The present study was undertaken to investigate the antifungal, antiaflatoxigenic, antioxidant, and anticancer activities of *Glycyrrhiza glabra* L. and *Matricaria chamomilla* L. essential oils and their phytochemical composition. The essential oils were obtained by hydrodistillation and their phytochemical composition was determined throughout gas chromatography-mas spectrometry (GC-MS) analysis. Both essential oils and their mixture showed broad antifungal spectrum against *Aspergillus flavus*, an important food contaminating fungus. The essential oils of *G. glabra* and *M. chamomilla* completely inhibited aflatoxin B1 (AFB1) production at 800 ppm. Both oils exhibited antioxidant activity as DPPH free radical scavenger in dose dependent manner. Percentage of radical scavenging activity of *G. glabra* and *M. chamomilla* oils at 400 µg/ml were calculated to be 85.2 and 91.7%, respectively as compared to standard (BHT) with 75.6% activity at the same concentration. The anticancer properties of essential oils against cells (MCF-7) were evaluated. In anticancer activity exposure of essential oils caused a significant decrease in cell viability in MCF-7 cell line (breast carcinoma). Exposure of MCF-7 cells with *G. glabra* essential oils resulted in dose dependent increase in cell growth inhibition (CGI) varying from 3 to 77% at concentration ranging from 10 to 640 µg/ml. Similarly, 7 to 89% CGI was obtained when *M. chamomilla* essential oils was used. The present study demonstrated that essential oils of *G. glabra* and *M. chamomilla* have potent antifungal, antioxidant, and anticancer with the presence of effective phytochemicals.

Key words: *Glycyrrhiza glabra*, *Matricaria chamomilla*, essential oil, antifungal, antiaflatoxigenic, antioxidant, anticancer.

INTRODUCTION

Plants and their essential oils are potentially valuable sources of antimicrobial compounds. Several studies have been published on the antimicrobial activities of plant compounds against many different types of microorganisms, including food-borne pathogens (Friedman et al., 2002; Tassou et al., 1995; Rančić et al., 2005). The main components of essential oils mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones are accountable for the biological activity of aromatic and medicinal plants as well as for their fragrance. Owing to these properties, spices and herbs have been added to food since ancient time, not only as flavoring agents but also as preservatives (Kalemba and Kunicka, 2003).

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There is a growing awareness that biological contaminants in food stuffs may play an etiological role in various human diseases (Peshin et al., 2002). Food safety and security is a health and economic concern and cannot be taken lightly especially in Africa where 80% of the population depends on agriculture for living. Over the years, there have been outbreaks of food poison sometimes resulting in loss of life. It has been estimated that as many as 30% of people in industrialized countries suffer from food borne diseases each year (WHO, 2000). Microorganisms play a major role in contamination of stored food deteriorating quantitatively and qualitatively. Fungi are momentous destroyer of foodstuffs during storage, rendering them unfit for human consumption by hindering their nutritive value and sometimes by producing mycotoxins (Singh et al., 2010). Mycotoxins are natural contaminants of cereals and other food commodities throughout the world and they drastically impact human and animal health. Mycotoxins are toxic substances produced mostly as secondary metabolites by filamentous fungi that grow on seeds, grains, and feed in the field, or in storage. Among the mycotoxins, aflatoxins comprise a group of chemically diverse compounds originating from secondary metabolism of filamentous fungi especially *Aspergilllus flavus* and *Aspergilllus parasiticus.* Aflatoxins are stable under normal food processing conditions and can therefore be present not only in food and feed, but also in processed products and threaten both human and animal health as they are known to be carcinogens, (Nizam and Oguz, 2003; Omidbeygi et al., 2007; Sidhu et al., 2009). These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Park et al., 2004; Egal et al., 2005). Among all classes of aflatoxins, aflatoxin B1 (AFB1) is known to be the most significant in terms of animal and human health risk (Coulombe, 1993).

Physical, chemical and biological methods have been investigated in order to prevent the growth of aflatoxin producing fungi and to eliminate or reduce the levels of aflatoxins or to degrade or detoxify aflatoxins in foods and feeds (Thanaboripat, 2002). Extracts and powders of various spices, herbs and essential oils have been reported to have antimicrobial activity against aflatoxin producing fungi and some of them also inhibit aflatoxin formation (Thanaboripat et al., 1989; Masood et al., 1994; Prasad et al., 1994; Thanaboripat et al., 1997; Thanaboripat et al., 2002; Thanaboripat, 2003; Krishnamurthy and Shashikala, 2006). Many essential oils have also been reported as effective inhibitors of fungal growth and aflatoxin production (Mahmoud, 1994; Razzaghi-Abyaneh et al., 2008). Great success has been achieved to reduce mycotoxigenic fungi and mycotoxins in foods using plant products such as plant extracts and plant essential oils (Reddy et al., 2010). Many studies have shown that natural antioxidants from plant sources can effectively inhibit oxidation of food and reduce the risk of age-dependent diseases (Santhi et al., 2011; Zou et al., 2004). The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds (Ivanova et al., 2005). The number of reports on the isolation of natural antioxidants, mainly of plant origin, has increased immensely during the last decade (Djeridane et al., 2007).

Cancer is a general term applied to malignant diseases described by quick and uncontrolled abnormal cells formation which can mass together to form a growth or proliferate throughout the body and it may progress until it causes death. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Medicinal herbs have been widely used for treatment of diseases in traditional way for several generations. An interaction between traditional medicine and modern biotechnological tools is to be established towards new drug development. The interference between cell biology, *in vitro* assays and structural chemistry will be the best way forward to obtain valuable leads. There is considerable scientific evidence to suggest that nutritive and non nutritive plant-based dietary factors can inhibit the process of carcinogenesis effectively (Aboul-Enein et al., 2012).

In this context, the present paper reports on antifungal, antiaflatoxigenic, antioxidant, and anticancer activity of essential oils of *Glycyrrhiza glabra* roots and flowers of *Matricaria chamomilla* and their combination. The phytochemical composition of essential oils was also studied. It may be noted that *G. glabra* is used in traditional medicine to treat liver diseases and is a major component of polyherbal formulations for the cure of hepatotoxicity (Rajesh et al., 2000), it was also reported to have hyporcholesterolemic and hypoglycemic activities (Sitohy et al., 1991) and to have protective role against oxidative stress (Haraguchi et al., 2002). *M. chamomilla* has been used for centuries as a medicinal plant mostly for its anti-inflammatory, anaglestic, antimicrobial, antispasmic and sedative properties (McKay et al., 2006; Gardiner, 2007).

MATERIALS AND METHODS

Isolation and identification of fungal species from food samples

Sampling of food commodities was performed according to the method provided by Bainton et al. (1980). Representative samples were randomly collected from field, market and storage facilities between June, 2012 and August, 2012. Field samples were randomly collected from farms just before harvest. Contents of traditional storage facilities and sacks in the markets were thoroughly mixed and samples were taken from many points of the facilities. Between 250 and 500 g of each sample of the various food commodities were collected. The samples were put in sealed polythene bags and transported to our microbiology laboratory for isolation, identification and characterization. Two different culture

media were prepared; Potato-Dextrose-Agar and Czapek media were found suitable for growth and sporulation of most fungi. The isolation of mycoflora and its pure culture were performed under aseptic conditions. Three replicates of each petri plate of pure culture were incubated at 28°C for 7 days. The grown colonies were identified according to authentic manuals of fungi (Gilman, 1957; Barnett and Hunter, 1972; Samson and Reenen-Koekstra, 1988; Moubasher, 1993; Kern and Blevins, 1997). The toxigenic strain of *A. flavus* was chosen as test fungus for the present study.

Plant material

G. glabra roots and *M. chamomilla* flowers were collected in August, 2012 from SEKEM'S company farms at Belbes, 60 km North Cairo, Egypt. The samples were selected from the field after harvest and authenticated at Botany Department Herbarium, Faculty of Science, Cairo University where voucher specimens have been deposited.

Extraction of essential oils

The essential oils were extracted from the plant powder by hydrodistillation using the Egyptian Pharmacopoeia (Egyptian Pharmacopoeia, 1984). The distillate was extracted with diethyl ether after saturation with sodium chloride. The ether extract was dehydrated over anhydrous sodium sulfate; solvent was removed under reduced pressure at 20°C. An oil mixture was prepared using 1:1 (v/v) ratio of both essential oils. Essential oils were stored at 4°C in airtight containers prior to analysis by gas chromatography-mass spectrometry (GC/MS).

Identification of essential oils constituents using GC/MS

GC-MS analysis was performed using SHIMADZU Q p5050A, GC/MS-5989B. The GC column was DBI (30 m × 0.53 mm × 1.5 µm) fused silica capillary column. The GC conditions were as follows: Carrier gas: Helium (flow rate 1ml/min), Ionization mode: EL (70eV), Temperature program: 40°C (static for 2 min), and then gradually increasing (160°C at a rate of 2°C min^{-1}) up to 250°C (static for 7.5 min); detector and injector temperature at 250°C. After stabilization of the chromatographic conditions, the samples were injected and mass spectrum of each peak was determined. The equivalent compound name, molecular weight and structure was concluded from the G1035A Wiley PBM Library (Probability Based Matching) of GC-MS. Qualitative identification of the essential oils was achieved by library searched data base Willey 275LIB and by comparing their retention index and mass fragmentation patterns with those of the available references and with published data in literature (Adams, 2007). The percentage composition of volatile oil components was determined by computerized peak area measurements.

Antifungal activity assay

Antifungal activity is determined by poisoned food technique (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapliyal, 2002). Five-day old culture of *A. flavus* was punched aseptically with a sterile cork borer of generally 7 mm diameter. The fungal discs were then put on Czapek's-dox agar (CDA) plates (90 mm). The agar plates have been prepared by impregnating different concentrations of essential oils and their combination (200, 400, 600, 800 and 1000 µg/ml) at a temperature of 45 to 50°C. The control plates were prepared using sterilized distilled water in place of essential oil. The plates were then incubated at temperature 28 ± 2°C. Colony diameter was recorded by measuring the two opposite circumference of the colony growth. Percentage inhibition of mycellial growth was evaluated by comparing the colony diameter of poisoned plate (with essential oil) and non-poisoned plate (with distilled water) and calculated using the following formula:

Mycelial inhibition (%) = Mycellial growth (control) – Mycellial growth (treatment) / Mycellial growth (control) × 100

Efficiency of essential oils in inhibition of aflatoxin production

The method described in literature by Sinha et al. (1993) was used for the determination of concentration of aflatoxin B_1 (AFB_1). Different concentrations of essential oils and their mixture (100, 200, 400, 600, 800, and 1000 ppm) were prepared by dissolving their requisite amount in 0.5 ml acetone and then mixed with 24.5 ml SMKY broth medium in 100 ml flask. The control was prepared without essential oil. Flasks were inoculated with 0.5 ml spore suspension (10^6 ml^{-1}) prepared in 0.1% tween 80 and incubated at 28°C for 12 days. The content of each flask was filtered through Whatman filter paper No. 1 and the filtrate was extracted with 20 ml chloroform in a separating funnel. The chloroform extract was evaporated on water bath at 70°C and redissolved in 1 ml chloroform. 50 µl of extract was spotted on TLC plate (20 × 20 cm of Silica gel-G) and developed in toluene:isoamyl alchol:methanol (90:32:2; v/v/v). The intensity of AFB_1 was observed in UV transilluminator at 360 nm. Spots of AFB_1 on TLC were scraped and dissolved in 5 ml methanol, centrifuged at 3000 rpm (5 min) and optical density of supernatant was recorded at 360 nm using spectrophotometer (Singh et al., 2010). The amount of AFB_1 was calculated according to Singh et al. (2008):

$$AFB_1\ (\mu g/kg) = \frac{D \times M}{E \times L} \times 1000$$

D = optical density, M= molecular weight of AFB_1 (312), E= molar extinction coefficient (21,800), and L= path length (1 cm cell was used).

Antioxidant activity of *G. glabra* and *M. chamomilla* essential oils

The scavenging effect of 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical was measured by the method in literature (Chou et al., 2009). 5 µl of different concentrations of essential oils of *G. glabra* and *M. chamomilla* (50 to 400 µg/ml) were separately incubated with 95 µl of a 0.3 mM DPPH methanol solution. The absorbance of each solution after 30 min incubation was measured at 517 nm against a blank of butylated hydroxyl toluene (BHT). The DPPH radical scavenging activity was expressed as the inhibition percentage calculated as:

Inhibition percentage (%) = (Absorbance of control - Absorbance of sample) / Absorbance of control ×100

Cell lines and culture

Human breast carcinoma (MCF-7) cell line was obtained from National Cancer Institute (Cairo, Egypt). Cells were cultured as monolayer in RPMI 1640 medium with 5% FBS and 1% penicillin-streptomycin cocktail at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2.

Measurement of cell viability by MTT assay

The effect of essential oils of *G. glabra* and *M. chamomilla* (10 to 600 µg/ml) on the viability of cells was determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazoliumbromide (MTT) assay. The cells were plated at 1×10^5 cells per well in 200 µl of complete culture medium containing 10 to 600 µg/ml concentrations of freeze dried essential oils in 96-well micro titer plates. Each concentration of essential oils was repeated in five wells. After incubation for desired times at 37°C in a humidified incubator, cell viability was determined. 50 µl MTT was added to each well and incubated for 2 h after which the plate was centrifuged at X 600 g for 5 min at 4°C. The MTT solution was removed from the wells by aspiration. After removal of the medium, 0.1 ml of buffered DMSO was added to each well, and plates were shaken. The absorbance was measured on a microtiter plate reader (Tecan, Switzerland) at the wavelength of 540 nm.

Statistical analysis

All measurements were repeated three times for each treatment and the data were reported as mean ± standard error (SE). The data were also statistically analyzed using One-way analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.05$ using Duncan's multiple range test (by SPSS, 16.1 Chicago, USA).

RESULTS AND DISCUSSION

Identification of essential oil constituents

The yield of *G. glabra* and *M. chamomilla* essential oils was 5.8 and 13.4 ml/kg, respectively. The essential oil of *G. glabra* was yellow in color. GC-Mass chromatography of *G. glabra* essential oils revealed the presence of the following compounds (α–pinene, β-pinene, octanol, γ-terpinene, stragole, isofenchon, β-caryophyllene, citronellyl acetate, caryophyllene oxide and geranyl hexanolate) (Table 1). It is obvious that geranyl hexanolate represented the highest percentage up to (34%) while β-pinene was the lowest one up to 1.7%, whereas the essential oils of *M. chamomilla* was sticky, blue in color with characteristic odor. GC-MS analysis of essential oils identified eleven compounds (germacene D, bicyclogermacrene, β-farnesene, α-bisabolol oxide B, α-bisabololo, chamazulene, α-bisabolol oxide A, guaizulene, cis-z-α-bisabolene expoxide, cis-ene-yne-dicycloether, trans-ene-yne-dicycloether) (Table 2). It is clear from the data that α-Bisabolol oxide A and Chamazulene are the major compounds which accounted for 29.4 and 27.5, respectively.

The oils were standardized by chemical profile. The composition of essential oils fluctuates with respect to ecological and geographical condition, age of plant and time of harvesting (Bagamboula et al., 2004). Differences among chemical compositions of the essential oils widely depend on production conditions such as climate, soil, harvest date, storage time, variety and cultivar factors (Blair et al., 2001) and thus different chemotypes of particular essential oil have been documented in literature. Such variation in phytochemical composition of essential oils would beyond doubt modify their biological activity. Therefore, authentication of phytochemical composition of essential oil is significant before suggesting it for antimicrobial activity.

Table 1. Phytochemical composition of essential oil of *G. glabra*.

Peak No.	RT[a]	Compound	Percentage
1	4.13	α-Pinene	4.2
2	4.3	β-Pinene	1.7
3	6.11	Octanol	5.1
4	7.23	γ-Terpinene	12.5
5	7.8	Stragol	9.5
6	9.6	Isofenchon	16
7	13.55	β-Caryophyllene	7.7
8	14.03	Citronellylacetate	4.1
9	14.33	Caryophylleneoxide	5.1
10	14.88	Geranylhexanolate	34

[a]RT – retention time.

Isolation and identification of fungal species

This study shows that all sampled food commodities were found to be contaminated by fungi to various degrees. Fourteen fungal species belong to five terrestrial fungal genera were isolated from various food commodities in field, storage sacks, and market (Table 3). *Aspergillus* was the leading genus where the most dominant fungal species in all tested food samples was *A. flavus*. Food samples obtained from field were the most infested of the three samples assayed while stored food samples were slightly more infested than marketed samples which were the least infested with fungi.

Our results went parallel with those obtained earlier by Amadi and Adeniyli (2009) who showed that a total of eight fungal species comprising five different genera were isolated from stored maize, rice and millet grains. Four of these fungi were different species of the genus *Aspergillus*. Similarly, Agrios (1978) found that the mostcommon storage fungi are *Aspergillus* and *Penicilium* species; Amadi and Oso (1996) reported that *Aspergillus* spp., *Mucor hiemalis*, *Macrophomina phaseolina*, *Rhizopus oryzae*, *Alternaria longissima*, *Cochliobolus pallescens*, *Botryodiplodia theobromae* and *Colletotrichum* species are found in *Vigna unguiculata* seeds in Ibadan, Nigeria. Amadi (2002) also accounted for eleven different fungal species including *Alternaria*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium,* and *Mucor* species in *Saccharum officinarum* seeds.

In a study carried out (Makun et al., 2010) to assess the incidence of fungi and mycotoxins contamination of some Nigerian cereals and powder. They found that all

Table 2. Phytochemical composition of essential oil of *M. chamomilla.*

Peak No.	RT[a]	Compound	Percentage
1	18.7	Germacene D	2.5
2	21.4	Bicyclogermacrene	1.7
3	22.4	β-Farnesene	6.2
4	28.7	α-Bisabolol oxide B	1.2
5	30.4	α-Bisabololo	9.3
6	35.5	Chamazulene	27.5
7	36.2	α-Bisabolol oxide A	29.4
8	37.2	Guaizulene	5.1
9	37.6	Cis-z- α-Bisabolene expoxide	9.8
10	40.61	Cis-ene-yne-Dicycloether	4.0
11	42.5	Trans-ene-yne-Dicycloether	3.3

[a]RT – retention time.

Table 3. Total count (TC), number of cases of isolation (NCI) and occurrence remarks (OR) of fungi isolated from various food commodities from field, sack and markets on Potato-Dextrose-Agar and Czapek media.

Fungal genera and species	Field			Sack			Market		
	TC	NCI	OR	TC	NCI	OR	TC	NCI	OR
Aspergillus	163	-	-	63	-	-	45	-	-
A. flavus	38	5	H	23	5	H	20	5	H
A. niger	27	4	M	18	4	M	10	4	M
A. fumigatus	25	4	M	12	4	M	11	1	R
A. terreus	24	3	L	-	-	-	-	-	-
A. glaucus	15	2	R	-	-	-	-	-	-
A. nidulan	14	3	L	-	-	-	4	1	R
A. parasiticus	10	2	R	10	3	L	-	-	-
A. versicolor	10	1	R	-	-	-	-	-	-
Penicillium	50	-	-	30	-	-	12	-	-
P. expansum	30	3	L	30	1	R	12	3	L
P.citrinum	20	2	R	-	-	-	-	-	-
Alternaria	10	-	-	25	-	-	4	-	-
A. alternata	10	1	R	25	2	R	4	2	R
Rhizopus	20	-	-	22	-	-	15	-	-
R. oryzae	15	4	M	-	-	-	-	-	-
R. stolonifer	5	1	R	22	1	R	15	4	-
Fusarium	22	-	-	15	-	-	-	-	-
F. oxysporum	22	5	H	15	4	M	-	-	-
Total count	265	-	-	155	-	-	76	-	-
Number of species	14	-	-	8	-	-	7	-	-

1–2: Rare, 2–3: Low, 3–4: Medium, 4–5: High.

the grain samples contained more than one species of fungi with maize having more fungal infestation than dry yam chips. It was observed in boiled rice, that medium of food contained more complex fats and carbohydrates which is an excellent growth medium that prompted the growth of various diverse types of mycoflora. Among the

Table 4. Antifungal efficacy *of G. glabra*, *M. chamomilla* essential oils and their oil combination on *A. flavus* on Czapek's-dox agar medium

Treatment (µg/L)	Diameter (mean ± SE) of mycelial growth (mm) including disc diameter of 7 mm						
	0 day	2 days	4 days	6 days	8 days	10 days	I* (%)
Control	7.0±0.0^{a}	11.5±0.1^{g}	23.4±0.2^{h}	34.5±0.2^{j}	51.9±0.4^{j}	80.7±0.3^{j}	-
G. glabra (200)	7.0±0.0^{a}	9.5±0.25^{e}	13.5±0.2^{f}	29.7±0.15^{h}	39.8±0.4^{h}	60.2±0.1^{h}	25.4
G. glabra (400)	7.0±0.0^{a}	8.3±0.25^{d}	8.7±0.15^{c}	21.3±0.15^{e}	28.7±0.05^{e}	39.1±0.2^{e}	51.5
G. glabra (600)	7.0±0.0^{a}	7.4±0.1^{ab}	7.8±0.15^{b}	17.8±0.3^{c}	16.3±0.15^{b}	30.3±0.35^{c}	62.4
G. glabra (800)	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	100
G. glabra (1000)	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	100
M. chamomilla (200)	7.0±0.0^{a}	10.8±0.25^{g}	16.7±0.25^{g}	32.5±0.2^{i}	43.4±0.2^{i}	65.4±0.2^{i}	18.9
M. chamomilla (400)	7.0±0.0^{a}	10.4±0.4^{f}	10.8±0.25^{e}	25.8±0.4^{g}	33.7±0.3^{g}	43.2±0.35^{f}	46.4
M. chamomilla (600)	7.0±0.0^{a}	8.05±0.15^{cd}	9.3±0.15^{d}	20.7±0.05^{d}	20.3±0.2^{d}	36.3±0.15^{d}	55
M. chamomilla (800)	7.0±0.0^{a}	7.0±0.1^{a}	7.0±0.23^{a}	7.1±0.44^{a}	7.0±0.0^{a}	7.0±0.0^{a}	100
M. chamomilla (1000)	7.0±0.0^{a}	7.0±0.05^{a}	7.0±0.09^{a}	7.36±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	100
Oil mixture (200)	7.0±0.0^{a}	7.7±0.2^{c}	10.5±0.3^{e}	25.0±0.3^{f}	31.4±0.2^{f}	49.8±0.4^{g}	38.2
Oil mixture (400)	7.0±0.0^{a}	7.4±0.15^{ab}	7.3±0.4^{a}	16.3±0.4^{b}	19.8±0.3^{c}	28.6±0.3^{b}	64.5
Oil mixture (600)	7.0±0.0^{a}	7±0.3^{a}	7.0±0.3^{a}	7.0±0.07^{a}	7.0±0.09^{a}	7.0±0.11^{a}	100
Oil mixture (800)	7.0±0.0^{a}	7±0.11^{a}	7.0±0.22^{a}	7.0±0.13^{a}	7.0±0.01^{a}	7.0±0.28^{a}	100
Oil mixture (1000)	7.0±0.0^{a}	7±0.06^{a}	7.0±0.08^{a}	7.0±0.41^{a}	7.0±0.2^{a}	7.0±0.2^{a}	100

%I*= percentage of growth inhibition at day 10. Values are mean (n=3) ± SE. The means followed by same superscript letter in the same column are not significantly different according to ANOVA and Duncan's multiple range tests.

most abundantly found species, the prominent are *A. flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus clavate* and *Aspergillus ochraceus* (Yamazaki et al., 1970; Ueno and Ueno, 1972; Eskola et al., 2001). The presence of *A. flavus* species in raw materials and dry ready pet food has been reported (Campos et al., 2008). Mycological examination of feeds revealed the recovery of 180 isolates (36%) of *A. flavus* and 65 isolates (13%) of *A. parasiticus* from the various samples studied (Azab et al., 2005) whereas 200 isolates (56.33%) of *A. flavus* were recovered from 355 animal feed samples (Connole et al., 1981).

Antifungal efficacy of essential oils

Throughout antifungal assay, all tested concentrations of essential oils were found significantly effectual over control from day-2 to day-10 in accordance with ANOVA and Duncan's multiple range test (Table 4). It could be seen that as the oil concentration increases the inhibitory effect increases. Complete inhibition of growth was recorded at 800 and 600 µg/L of both essential oils and their mixture, respectively. Oil mixture was found most effective when compared with that of *G. glabra* or *M. chamomilla* when used separately. At 400 µg/L, the growth of *A. flavus* was inhibited at 54.5, 46.4, and 64.5% against essential oil of *G. glabra*, *M. chamomilla*, and their combination, respectively.

Our results are consistent with those of Tharkar et al. (2010) who found that the hydro alcoholic extract of *G. glabra* exhibited antifungal activity. Similarly in another report, *G. glabra* extracts and their fractions exhibited the most interesting inhibitory activities against *Candida albicans* and *Trichophyton rubrum* (Meghashri, 2009). Liquorice (*G. glabra*) extracts with 80% methanol (oil-based extract) was found to have high fungicidal effect against *Arthrinium sacchari* and *Chaetomium funicol* (Hojo and Sato, 2002). Glabridin, an active constituent of *G. glabra* roots, was found to be active against both yeast and filamentous fungi (Fatima et al., 2009). The antimicrobial activity of *G. glabra* may be attributed to alkaloid, saponins, flavonoides, tannin, glycosides and phenols. These phytochemical groups are well known antimicrobial compounds (Scalbert, 1991; Field and Lettin, 1992).

The date reported in this study are in agreement with those of who stated that *M. chamomilla* produces a volatile oil which is a valuable source of α-bisabolol oxides A and chamazulene which are the candidate molecules of antimicrobial activity of the essential oil (Bisht et al., 2012). In this context, the antifungal activity of *M. chamomilla* essential oil against *A. flavus* and *C. albicans* has been reported (Roby et al., 2012). *M. chamomilla* exhibiting higher fungistatic effect was observed in case of chamomile which leads to 95% inhibition of fungal growth at 3000 ppm (Soliman and Badeaa, 2002). The antifungal activity of *M. chamomilla* L. flower essential oil was documented against *A. niger* (Tolouee et al., 2010).

Table 5. Antiaflatoxigenic efficacy of *G. glabra*, *M. chamomilla* essential oils and their combination on mycelial biomass (g) and AFB1 production (µg/ml) of *A. flavus*.

Treatment	Concentration (µg/ml)	Biomass	AFB1 (µg kg^{-1})	I* (%)
Control	0.0	0.666±0.003^{g}	432.5±1.25^{h}	-
	200	0.522±0.001^{eg}	122.2±1.50^{g}	71.7
	400	0.329±0.004^{cde}	32.2±0.35^{d}	92.55
G. glabra	600	0.22 1±0.005^{abcd}	11.6±0.30^{b}	97.31
	800	0.153±0.00^{abc}	0.00±0.0^{a}	100
	1000	0.000±0.00^{a}	0.00±0.0^{a}	100
	200	0.543±0.00^{eg}	101.8±1.4^{f}	76.46
	400	0.411±0.004^{de}	21.2±0.1^{c}	95.09
M. chamomilla	600	0.202±0.004^{abcd}	4.1±0.25^{a}	99.05
	800	0.100±0.0^{cde}	0.00±0.0^{a}	100
	1000	0.000±0.0^{a}	0.00±0.0^{a}	100
	200	0.258±0.002^{bcd}	83.5±2.7^{e}	80.6
	400	0.091±0.004^{ab}	4.1±0.05^{a}	99.05
Oil combination	600	0.000±0.000^{a}	0.00±0.0^{a}	100
	800	0.000±0.0^{a}	0.00±0.0^{a}	100
	1000	0.000±0.0^{a}	0.00±0.0^{a}	100

%I*= percentage inhibition of aflatoxin B1. Values are mean (n=3) ± SE. The means followed by same superscript letter in the same column are not significantly different according to ANOVA and Duncan's multiple range tests.

Antiaflatoxigenic potential of essential oils

A direct relationship was observed between growth of *A. flavus* and AFB1 production, that is, a significant decrease in mycelial biomass resulted in low AFB1 production and vice versa. The essential oils of *G. glabra* and *M. chamomilla* completely inhibited AFB1 production at 800 µg/ml while oil mixture could inhibit at 600 µg/ml which indicate that there was synergism between the oil components when the two oils mixed together. At 800 µg/ml, growth was recorded in sets treated with *G. glabra* and *M. chamomilla* essential oils, but aflatoxin B1 production was completely inhibited (Table 5).

Essential oils can reduce the injured effect of aflatoxins by two different methods. Firstly, DNA binding formation of aflatoxins is reduced by essential oils. Secondly, aflatoxins result in an increase of reactive oxygen species (ROS) and essential oils react with ROS. Consequently, essential oils protect the cells from injurious impact of aflatoxins (Alposy, 2010).

Little is known about the efficacy of *G. glabra* and *M. chamomilla* oils as aflatoxin inhibitor. Licorice compounds exhibited a concentration-dependent inhibition of S-9-mediated mutagenesis induced by AFB_1. These compounds also significantly inhibited AFB_1 binding to DNA and significantly decreased the activation of AFB_1 to mutagenic/carcinogenic metabolites (Ngo et al., 1992). Chan et al. (2003) investigated the effect of glycyrrhizic acid (GA), a major component of licorice, on AFB1-induced cytotoxicity in human hepatoma cell line (HepG2). Increasing the liquorice content of the diet to 450 mg/kg essentially negated the effects of aflatoxin. The essential oil of *M. chamomilla* showed specific inhibition toward aflatoxin G_1 (AFG_1) production. The (*E*)- and (*Z*)-spiroethers, active components of oil, have inhibitory concentration of 50% (IC_{50}) values of 2.8 and 20.8 µM, respectively, without inhibiting fungal growth (Yoshinari, 2008).

Antioxidant activity of essential oils

The change in absorbance produced by reduced DPPH was used to evaluate the ability of essential oils to act as free radical scavengers. Both essential oils exhibited DPPH radical scavenging activity in dose dependent manner (Figure 1). Essential oils of *G. glabra* and *M. chamomilla* at concentration of 400 µg/ml exhibited 85.2 and 91.7%, respectively when compared with the standard (BHT) with 75.6% activity at the same concentration.

The antioxidant properties of medicinal plants rely on the plant, its variety, environmental conditions, seasonal and climatic variations, geographical regions of growth, degree of maturity, growing practices, and numerous other factors such as postharvest treatment and processing. Additionally, composition and concentration of the present antioxidants, such as phenolic compounds,

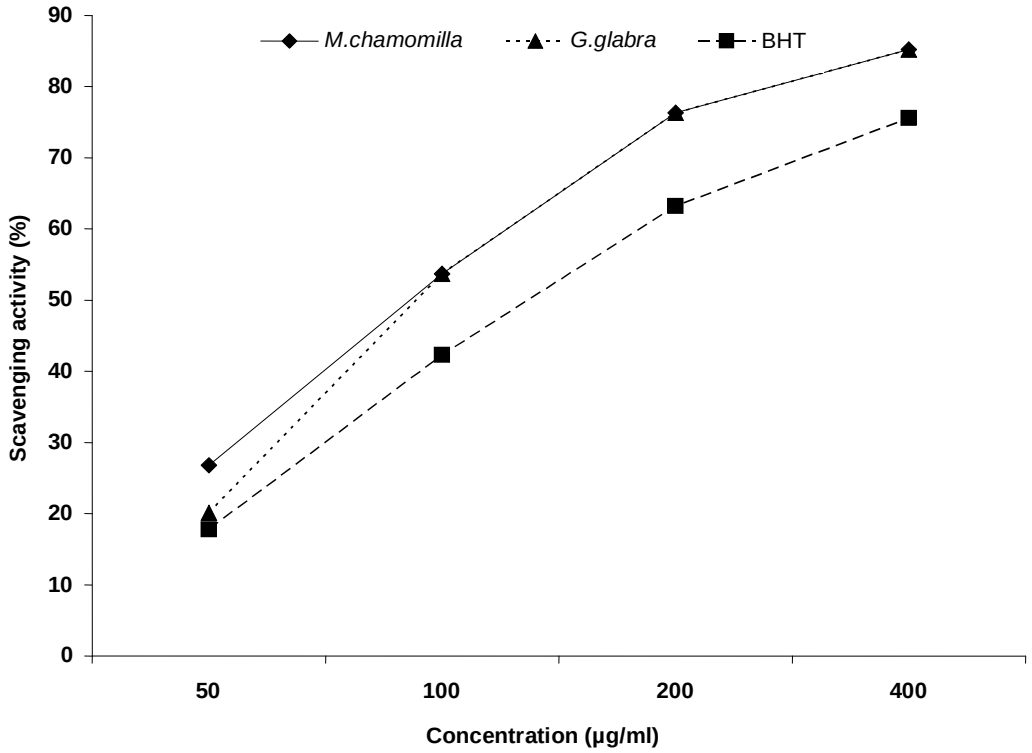

Figure 1. Radical scavenginging activity of BHT and essential oils of *G. glabra* and *M. chamomilla* at various concentrations.

are related to antioxidant activity (Škrovánková et al., 2012).

The results of this study are in agreement with those in which crude methanolic extract of *G. glabra* at concentration 62.5 µg exhibited 91.3% scavenging activity (Lateef et al., 2012). Similarly, Hong et al. (2009) concluded that *G. glabra* polysaccharides (GGP) treatment can enhance immune activities, and reduce oxidative stress in high-fat mice. Ethyl acetate extract of *G. glabra* leaves have been proved to have antioxidant, anti-genotoxic and anti-inflammatory activities (Siracusa et al., 2011). Roots of *G. glabra* are a potential source of antioxidants and constituents which may be considered as leading compounds in drug discovery (Lateef et al., 2012). Singh et al. (2010) concluded that the extract of *G. glabra* have significant amount of phytochemicals and antioxidant enzymes.

The data presented in this study are consistent with those of Abdoul-Latif (2011) that reported that the IC_{50} value of essential oil and methanol extract of *M. chamomilla* were respectively 4.18 and 1.83 µg/ml. Antioxidant activity of essential oil from *M. Chamomilla* have been reported (Owlia, 2007). Chamomile extract could be used as natural antioxidant in food stuffs as complementary material (Sazegar et al., 2010). The antioxidant activity of *M. chamomilla* essential oils is mainly due to chamazulene and guaiazulene (Rekka et al., 1996).

Anticancer properties of essential oils

The MTT assay is an uncomplicated and consistent technique to determine cell viability used for screening of anti-proliferative agents. Figure 2 shows the summarized plots of cell growth inhibition (CGI) (%) versus concentrations of essential oils. Both *G. glabra* and *M. chamomilla* essential oils display anti-proliferative effects against the MCF-7 in dose-dependent manner. It was found that the higher the concentration of essential oil, the higher the percentage inhibition of cell proliferation percentages was. Overall, essential oils of *G. glabra* and *M. chamomilla* were found to inhibit the proliferation of the cell line. Exposure of MCF-7 cells with *G. glabra* essential oils for 24 h results in a cell dependent increase in CGI varying from 3 to 77% at concentration ranging from 10 to 640 µg/ml. Similarly, 7 to 89% CGI was obtained when *M. chamomilla* essential oil was used.

The results of this study are similar to those in which *G. glabra* extract and glycrrhizin, in *G. glabra* inhibit proliferation of breast cancer MCF-7 cells (Dong et al., 2007). Cytotoxic activity exhibited by methanolic extract of *G. glabra* clearly indicates the presence of plant bioactive principles of this extract which might be very useful as antiproliferative, antitumor and other bioactive agents (Meyer et al., 1982; Rahman et al., 2008; Hossain et al., 2004).

The results of this study are in harmony with those in

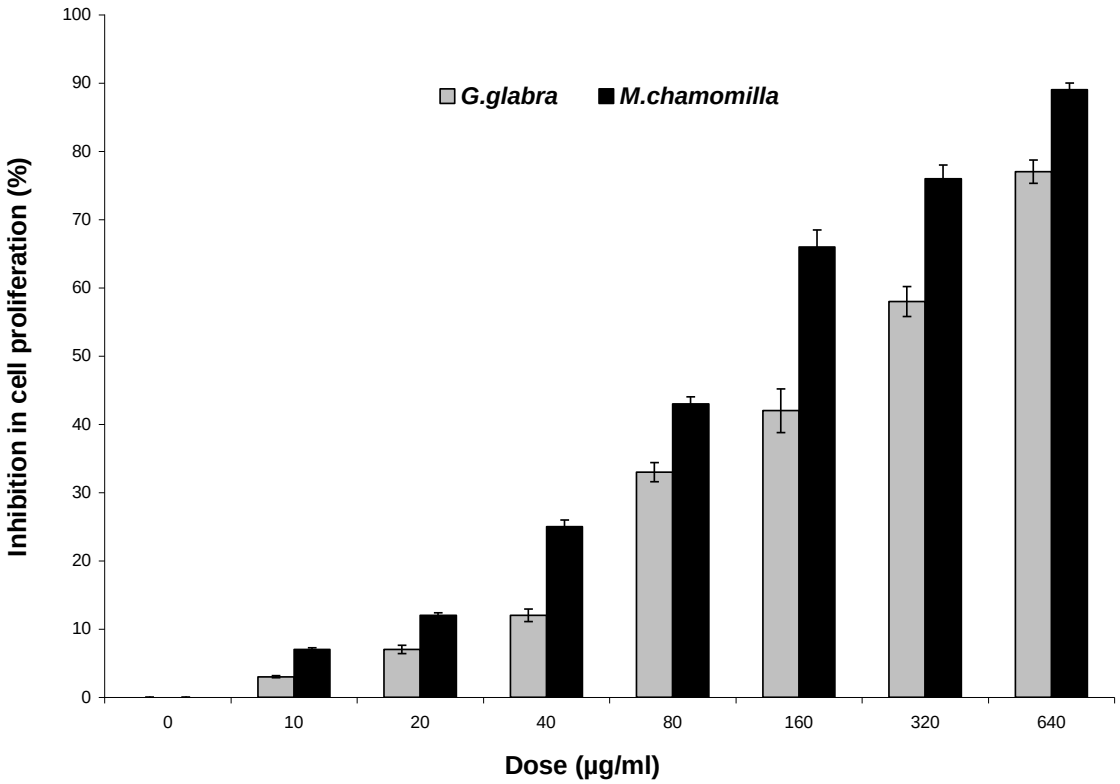

Figure 2. Effect of *M. chamomilla* and *G. glabra* EOs on cell growth inhibition of breast carcinoma cells MCF 7. Values represent mean± SE of 3 different assays at p< 0.01.

which the exposure of human prostate cancer PC-3 cells to both aqueous and methanolic chamomile extract caused induction of cell growth inhibition and apoptosis (Srivastava and Gupta, 2009). Matic et al. (2012) demonstrated that chamomile exert selective dose-dependent cytotoxic action against target cancer cells. α-Bisabolol, a major component of chamomile essential oil, induced a decrease in cell proliferation and viability in pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA, Paca2). The apoptotic effect of a-bisabolol, a sesquiterpene present in chamomile, against human liver carcinoma cell line HepG2 (Chen et al., 2010).

Conclusions

On the basis of the findings in this investigation, the essential oils of *G. glabra* and *M. chamomilla* may be suggested as plant based antimicrobial in addition to antioxidant food additive for improvement of shelf life of stored food commodities. It has also become obvious that these essential oils may supply efficient anticancer therapeutics. Such essential oils should be more broadly used in developing countries for avoidance and treatment of hazardous diseases such as cancer. The essential oils should be considered as important sources for drug discovery.

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