

Antioxidant and antiviral activities of some traditionally used herbs and spices in Egypt

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ABSTRACT

There is abundant evidence that a great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. With this respect a particular interest has been directed to search about antiviral agents. The alcoholic and volatile oils extracts of traditionally used nine plants (green tea, ginger, celery, basil, rosemary, cinnamon, peppermint, chamomile and black pepper) were evaluated for their in-vitro antioxidant and antirabies activities. The antioxidant assay revealed that all extracts have antioxidant activity (AOA), in the range of (0.96-4.02 mM trolox). The major compound which may be responsible for AOA in each extract was characterized by GC-MS. Different magnitudes of cytotoxicity and viral potency were detected among the tested plant extracts. The most potent antirabies activity was observed with ginger and basil crude extracts; chamomile and cinnamon volatile extracts which inhibit virus infectivity by 100 % at 500, 500, 0.1, and 0.1µg / ml, respectively without showing cytotoxic effect. This antirabies activity was confirmed by the variation in sensitivity to different extract concentrations. These results confirm the importance of these herbs and spices, and regard to their antiviral activities. So, further in-vivo research is needed to be combined with in-vitro data for adequate assessment.

Key words: Antiviral, medicinal plants, antioxidants.

INTRODUCTION

Serious attention is now paid to the cytotoxicity of active oxygen free radicals which are believed to cause various pathological conditions. Free radicals produced from unsaturated fatty acids and various metabolisms are well known to cause histotoxicity and promote the formation of additional free radicals in a chain reaction type manner. It is thought that, if the *in-vivo* activity of enzymes or free radicals scavengers is not high enough to inhibit free radicals,

various diseases such as arteriosclerosis, liver diseases, diabetes, inflammation, renal failure, cancer or accelerated aging may result (Niki, 1995).

Oxygen radicals, which are generated by the host's immune system during invasion by bacteria and microorganisms, have been shown to possess antimicrobial effect and to enhance the immune system in eliminating pathogens (Badway and Karnovsky, 1980). However, oxygen radicals are not necessarily beneficial to the host during viral infection, as they can cause pathological effects (Akaike *et*

al., 1998). A previous study showed that the amount of free radicals in alveolar phagocytic cells of influenza-infected mice was significantly increased when compared with noninfected control mice (Oda *et al.*, 1989). The survival rate of the influenza-infected mice was improved by injection of pyran copolymer-conjugated SOD, which served as a free radical scavenger. This finding suggests that compounds which exhibit antioxidant activity could reduce viral infection.

Introduction of synthetic antioxidants have caused some problems due to their highly volatile nature, instability and strict low restriction, etc, (Dapkevicins *et al.*, 1998). Which coupled with the problem of viral latency and conflicting efficacy in recurrent infection in immunocompromised patients (Field and Biron, 1994)? Ethnopharmacology provides an alternative approach for the discovery of antioxidant and antiviral agents; namely the study of medicinal plants with the history of traditional use as a potential source of substances with significant pharmacological and biological activities (Vlietinck *et al.*, 1991).

Rabies is an acute highly fatal infectious disease affecting all warm blooded animals and man. It is usually transmitted through biting of rabid animals to healthy man where the causative virus is often present in the saliva of the victim. Rabies is usually manifested by fatal encephalomyelitis, sign of mania irritation, ascending paralysis and ends with death (Bear, 1975). Rabies is of world wide distribution; regarding Egypt, it is enzootic in jackals and common in dogs (El-Kanawati, *et al.*, 2000).

Focusing attention on the plants medicinally used by indigenous people is the most efficient way to identify plants that may contain bioactive substances (Smith and Winder, 1996). Since prehistoric times, herbs have also been the basis for nearly all

medicinal therapy until synthetic drugs were developed in the 19th century. However, herbs and spices have also been identified as sources of various phytochemicals, many of which possess important antioxidant activity (Larson, 1988). The present study was conducted to elucidate the antioxidant and antiviral properties of the extracts from some traditionally used medicinal herbs against rabies virus infectivity.

MATERIALS AND METHODS

Plant materials

Dried green tea leaves (*Camellia sinensis*), ginger rhizomes (*Zingiber officinale*), celery seeds (*Apium graveiense*), basil leaves (*Ocimum basilicum*), rosemary leaves (*Rosmarinus officinalis*), cinnamon leaves (*Cinnamomum zeylanicum*), peppermint leaves (*Mentha piperita*), chamomile flowers (*Camomilla recutita*), and black pepper fruits (*Piper nigrum*) were purchased from local market and identified at the Department of Botany, Faculty of Science, Ain Shams Univ., Egypt.

Cell culture and virus

Baby hamster Kidney cell line (BHK-21) was cultured using Eagle's minimum essential medium supplemented with 5% fetal bovine serum. These cells were used to estimate the cytotoxicity and antiviral activity of the tested samples. Cell culture attenuated rabies virus (ERA-strain) was propagated in BHK cell (Edries, 1994) and supplied by the Department of pet animal Vaccine Research, Veterinary serum and Vaccine Research Institute, Cairo. Virus titration was carried out using the microtiter technique according to Rossiter and Jesset (1982). It had a titer 10^7 TCID₅₀/ml.

Preparation of plant extracts

The respective dried parts of the nine plant species (100 gm sample) were ground to fine powder and extracted with ethanol-water (90-10) for 48 hr at 25 °C. The slurry was filtrated and washed with ethanol. The alcoholic extracts were concentrated under reduced pressure in a rotary evaporator at 55°C for 3 hr. All extracts were lyophilized, weighted, and dissolved into phosphate buffered saline of pH 7.2 and DiMethylSulphOxide (DMSO) 1:1 according to Vijayan *et al.* (2004)

The volatile oils compounds of these plants like: polyphenol for (green tea, ginger and chamomile); terpenoids for (celery, basil, rosemary, cinnamon and peppermint); alkaloids for (black pepper) were also extracted from the dried plant materials according to methods described by Hufford *et al.* (1993), Rao *et al.* (1993) and Then *et al.* (2003), respectively, then subjected for further analysis.

Analysis of the major chemical compounds

The gas chromatography-mass spectrometric (GC-MS) method was adapted to perform the analysis and identification of the major compounds of the extracts. Mass of each concentrate was analyzed using a flame ionization detector (FID) to determine the percentage of the total peak area of the component and solvent. The relative percentage of the component was calculated by dividing the peak area of each component / total peak area of extract X 100 (Lee and Shibamoto, 2001). A Finnigan GC method equipped with RTX 5MS 30m x 0.25 mm i.d column was used for analysis of total component in each extract. The linear velocity of the helium carrier gas was 40 cm/sec at a split ratio of 20: 1. The injector and detector temperature was 280°C. The oven temperature

was programmed from 40: 320 °C at 5 °C/ min and hold for 10 min.

Measurement of the antioxidant activity

The antioxidant capacity of the tested samples prepared at 100 mg/ml. (in triplicate) was evaluated by using Fe-EDTA complex which reacts with hydrogen peroxide by a Fenton type reaction, leading to the formation of hydroxyl radical (OH[•]). These reactive oxygen species degrade benzoate, resulting in the release of ThioBarbituric Acid Reactive Substances (TBARS). Antioxidant Activity (AOA) of the tested plant extract samples was measured spectrophotometrically and calculated in mM trolox where, causes suppression of TBARS production as described by Koracevic *et al.* (2001).

Cellular toxicity assay

Each extract was dissolved in PBS: DMSO 1:1 and diluted in Eagle MEM without serum to a final concentration of 500 mg/ml for the plant total extracts and 100 mg/ml for the volatile oil extracts. These extracts were sterilized by filtration using 0.22 µm milipore filter. The cytotoxicity assay was carried out using 0.1 ml cell suspension containing 10,000 cells seeded in each well of 96-well microtiter plate. Fresh medium containing 10 fold dilutions of the tested sample was added after 24 hr of seeding. Control cells were incubated without test sample and with DMSO. The microtiter plate was incubated at 37 °C with 5% CO₂ for 72 hr. The morphology of the cells was inspected daily for microscopically detectable alterations.

Determination of Minimum Inhibitory Concentration (MIC)

MIC, regards to the concentration giving the least viral inhibitory activity and below which there is no further inhibition. Serial ten diluted plant extracts (concentration in µg /

ml) were placed into the virus seeded plates. The plates were incubated at 37 °C with 5% CO₂ for 72 hour. The cells were examined by the microscope and the MIC of each sample was determined.

Determination of the antiviral activity

Confluent monolayer of BHK-21 cells in 96-well plates were inoculated 25 µl of rabies virus (100TCID₅₀) then overlaid with 25 µl of each non toxic concentration of the test plant extracts. The antiviral property was checked by the cytopathic effect (CPE) inhibitory assay as described by Hu and Hsiung (1989) and by the virus yield reduction assay (Cinatl *et al.*, 1997). The anti-rabies activity was determined by inhibition of CPE compared with untreated infected cells (control).

RESULTS

Ethanol as a common organic solvent was selectively used for the extraction of the total active components from the different parts of the nine tested plant spices (green tea, ginger, celery, basil, rosemary, cinnamon, peppermint, chamomile and black pepper) which belong to different plant families (Table 1). Further purified active chemicals (volatile oils) were obtained from the same plants using various extracted methods. Both yields of crude and volatile components seemed to be different according to the plant type (Tables 3 and 4), the chemical analysis of the volatile oil was carried out using GC-MS, where, mixed of different chemical compounds in an individual extract. In all volatile oil extracts showed certain common constituents but with different ratios, however among these different compounds in each extract, there is a predominant one. The relative percentage of these major compounds was estimated in each extract (Table. 2).

The antioxidant activity of various alcoholic and volatile oil extracts was studied *in vitro* where; the free radical scavenging or the inhibition of TBARS formation of each extract was compared with those of the used standard antioxidant. Comparing the mean antioxidant activity (AOA) of both alcoholic and volatile oil extracts, it was shown that it is approximately exhibited similar AOA except the ginger, black pepper and chamomile extracts (Fig. 1). The higher AOA was detected with basil crude extract (4.02 mM trolox), while ginger volatile oil extract has low AOA (0.96 mM trolox).

Cytotoxicity assay of all tested plant extracts was performed before studying the antiviral activity to ensure that the antiviral activity is not caused by cytotoxic effect of the tested sample toward the BHK. Five volatile oil extracts of ginger, basil, peppermint, celery, and black pepper showed cytotoxicity even at high dilutions against BHK-21 cells, while pepper mint and green tea crude extracts did not exhibit any toxicity at all (Tables 3 and 4).

These extracts of the nine medicinal herbs and spices were tested for their antiviral activity against rabies virus in the crude and volatile oil forms. The results showed that seven crude plant extracts have this activity at a non toxic concentration to cell line (Table 3). Most of these extracts have partial antirabies activity at low concentrations used. The crude extract of basil and ginger exhibited detectable inhibitory effect against rabies virus with 100% at the same concentration (500 µg/ml). Peppermint and black pepper crude extracts failed to show antiviral property at all.

On the other hand, the volatile oil extracts of chamomile and cinnamon showed 100% antirabies activity at the same concentration (0.1µg/ml); the same extracts showed partial activity at higher

concentrations (Table 4), although, rosemary and green tea exhibited low antirabies activity.

Table (1): Traditional use of plant species selected for antiviral antioxidant investigations.

Common name	Scientific name	Family name	Part used	Popular use **
Basil	<i>Ocimum basilicum</i>	Lamiaceae	Leaf	Flavor for foods and soup, aiding control blood pressure
Black pepper	<i>Piper nigrum</i>	Piperaceae	Fruit	Condiment used as carminative reducing stomach and intestinal gas, stimulate the heart and kidney activity
Celery	<i>Apium graviens</i>	umbelliferae	Seed	Flavor agent have a sedative properties, blood pressure lowering
Chamomile	<i>Camomilla recutita</i>	Asteraceae	Flower	Tea used as a digestive aid, antihistaminic
Cinnamon	<i>cinnamomum zeylanicum</i>	Lauraceae	Leaf	Flavoring for many foods and drinks, reduce pain and fever, and relieve nausea and indigestion
Ginger	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	Excellent drink for respiratory system and digestion, and relieve headache
Green tea	<i>Camellia sinensis</i>	Theaceae	Leaf	Tea of leaves used for heart disease and diabetes, control blood pressure
Peppermint	<i>Mentha piperita</i>	Lamiaceae	Leaf	Drink aid digestion and calms upset stomach, relieve muscle spasms, and alleviate symptoms of respiratory infection
Rosemary	<i>Rosmarinus officinalis</i>	Lamiaceae	Leaf	Flavor for foods and soup, memory enhancing, and strength the circulatory and nervous system

** These information were provided from the following web sites: herbs-spices.net, herbalgram.org

Table (2): Chemical characterization of the tested plant extracts using GC-MS.

Plant name	Class of Chemical	Major compound	Relative %
Basil	Terpenoids	Ocimenone	34.20
Black pepper	Alkaloids	Piperinol	58.60
Celery	Terpenoids	Myrecetine	10.66
Chamomile	Poyphenolic	Anthole	79.00
Cinnamon	Terpenoids	Cinnamtanin	45.00
Ginger	Poyphenolic	Cineole	56.50
Green tea	Flavenoids	Octanol	27.90
Peppermint	Terpenoids	Neomenthol	39.00
Rosemary	Terpenoids	Borneol	9.90

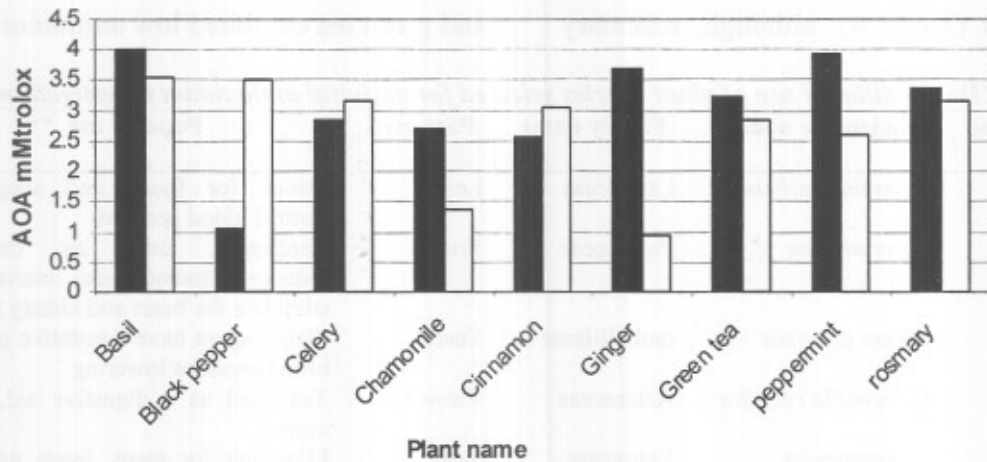


Fig. (1): Antioxidant activity (AOA) of the total crude extracts ■ and volatile oil extract □ of the nine tested plant species using (TBARS-assay).

Table (3): Cytotoxicity and antiviral activity of tested plant crude extracts.

Plant name	Yield % (W/W)	Cytotoxicity $\mu\text{g/ml}$	MIC $\mu\text{g/ml}$	Conc. used for antiviral activity ($\mu\text{g/ml}$)	Viral Inhibitory %
Basil	2.80	5000	0.5	500	100
				50	75
				5	25
Black pepper	3.90	500	-	50	-
				5	-
				0.5	-
Celery	2.30	5000	5	500	100
				50	50
				5	25
Chamomile	3.33	500	5	50	100
				5	25
				0.5	-
Cinnamon	3.20	50	5	5	75
				0.5	-
				0.05	-
Ginger	3.50	5000	0.05	500	100
				50	75
				5	50
Green tea	2.45	-	500	5000	100
				500	25
				50	-
Peppermint	2.95	-	-	50	-
				5	-
				0.5	-
Rosemary	3.80	500	5	50	100
				5	50
				0.5	-

* MIC: Minimum Inhibitory Concentration.

Table (4): Cytotoxicity and antiviral activity of selected plant volatile oil extracts.

Plant name	Yield % (W/W)	Cytotoxicity $\mu\text{g/ml}$	MIC* $\mu\text{g/ml}$	Conc. used for antiviral activity ($\mu\text{g/ml}$)	Viral Inhibitory %
Basil	0.07	toxic	-	-	-
Black pepper	0.09	toxic	-	-	-
Celery	0.02	toxic	-	-	-
Chamomile	0.04	100	0.0001	10 0.1 0.01	50 100 100
Cinnamon	0.03	10	0.01	1 0.1 0.01	50 100 -
Ginger	0.03	toxic	-	-	-
Green tea	0.02	1	0.001	0.1 0.01 0.001	0 25 -
Peppermint	0.02	toxic	-	-	-
Rosemary	0.09	1	0.0001	0.1 0.01 0.001	0 50 50

* MIC: Minimum Inhibitory Concentration.

DISCUSSION

Some of traditionally used plant species as flavoring agents of food or in folk medicine for primary health care were investigated for their antioxidant power and *in-vitro* antiviral activity against rabies virus. Simple test of the total antioxidant activity have been carried out to assess the presumable effect of these plant extracts. Both crude and volatile oil extracts exhibited antioxidant activity (AOA) with varying potentials where, ginger, basil, peppermint showed more potent AOA than rosemary, chamomile, and cinnamon crude extracts. Volatile extracts were arranged respecting to AOA in a descending order as black pepper, basil, rosemary, celery, then cinnamon. There is a positive association between the AOA and the plant antioxidant chemical components such as phenolics,

flavonoids, and terpenoids (Evans *et al.*, 1996). The chemical analysis of the volatile oil extracts was conducted using GC-MS, the major chemical compound in each extract could be responsible for the antioxidant activity like Ocimenene in basil, piperene in black pepper, cinnamtanin in cinnamon etc. Juliani and Simon (2002) analyzed the essential oil of basil including polyphenols and confirmed the close relation between the relative percentage of the basil chemical composition and the antioxidant activity. Lee and Shibamoto (2002) evaluated the antioxidant activity of volatile extracts of basil, rosemary, cinnamon, and chamomile and reported that AOA of the crude and volatile oil extracts was in the same descending order: Basil, rosemary, chamomile then cinnamon, which in agreement with the present data. They concluded that using two

independent assays for measuring AOA of these extracts revealed the presence of difference in sensitivity of the two assays. The potent AOA was highly attributed to the major chemical components in the volatile extracts which is also depending on the method of extraction and the solvent used. In extract of rosemary by stem distillation (essential oil extract) 1,8-cineole and borneol are different from those in solvent extract (carnosol and carnosic acid). Though carnosol and carnosic acid extract are potent antioxidant, 1,8 -cineole and borneol had not shown high antioxidant activity (Hopia *et al.*, 1996). This suggests the importance of developing and selecting a reliable assay for accurately measuring AOA of natural antioxidants.

The cytotoxicity and antiviral activity of the tested extracts showed that crude extracts were non-toxic at all. Moreover, seven out of nine extracts exhibited antirabies in a dose related manner. This is a credit to ethanol extraction as an organic solvent which will dissolve organic compounds better and hence liberates the active components required for antiviral activity. The volatile oil extracts had higher cytotoxicity for BHK-21 cell line like ginger, basil, celery, peppermint and black pepper too. Isamil and Aburjai (2004) found that the water extract of chamomile show higher antioxidant activity than the corresponding alcohol extract. This may explained why we used these herbs and spices as a tea drinks or food additives; it may minimize their cytotoxicity or maximize their AOA constituents. The lower antiviral activity of certain crude and volatile oil extracts could, in part due to the presence of antiviral compounds insufficient to inactivate all infectious virus particles or may be based on their interaction with proteins of cell surface and virus protein (Vijayan *et al.*, 2004).

Many studies investigated the antiviral activity of numerous medicinal plants (Abad *et*

al., 1999; Vijayan *et al.*, 2004). Nowadays, medicinal plants rich in antioxidant activity have the priority in this area as antiviral agents (Sokmen *et al.*, 2005; and Hamauzu *et al.*, 2005). In summary, it is concluded that these nine traditionally used herbs and spices in Egypt possess antioxidant activity; the crude extracts are more potent antiviral agents and less toxic than volatile oil extracts. Thus, these data suggest the viral therapeutic probability of these plants, but more investigations are required concerning the bioavailability and bioactivity of herbs and spices antioxidants.

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المخلص العربي

الأنشطة المضادة للأكسدة والفيروسات لبعض الأعشاب والتوابل الشائعة الاستخدام في مصر

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أثبتت الدراسات مدى ارتباط الأوكسجين الحر المنشطر بالأمراض الفيروسية، فكلما زاد تركيزه داخل الجسم زادت مضاعفات المرض كما أن نشاط الفيروس يؤدي إلى زيادة الأوكسجين الحر المنشطر داخل الجسم وأثر ذلك في العديد من الأمراض. لذلك أجرينا هذه الدراسة والتي تناولت بعض الأعشاب والتوابل شائعة الاستخدام في مصر لمعرفة احتوائها على مركبات مضادة للأكسدة ومضادة للنشاط الفيروسي. وهذه النباتات هي الريحان و الشاي الأخضر و القرفة و الزنجبيل و الكرفس و حصى لبان و النعناع و شيح البابونج و الفلفل الأسمر. وتم استخلاص محتويات هذه النباتات في صورتها الخام وصورتها الزيتية وقياس النشاط المضاد للأكسدة لهم جميعا وكذلك المحتوى الكيميائي باستخدام جهاز الكروماتوجرافي الغازي. ومعرفة ما لهم من تأثير مضاد للفيروس من خلال فيروس السعار داخل خطوط الخلايا المنزرعة. وأسفرت النتائج بالفعل عن وجود نشاط مضاد للأكسدة لجميع مستخلصات هذه النباتات بدرجات متقاربة، لذلك تم تعيين بعض المواد الكيميائية المختلفة بداخلها. وترتب على ذلك دراسة النشاط المضاد للفيروس وقد تم تحديد التأثير السام لهذه المستخلصات على الخلايا قبل دراسة تأثيرها على الفيروس. وبالرغم من عدم وجود تأثير سام للمستخلصات الخام خصوصا في التركيزات القليلة كان هناك تأثير سام لكثير من مستخلصات الزيوت الطيارة مثل للزنجبيل و الفلفل الأسمر و النعناع و الريحان و الكرفس (حتى التركيزات القليلة منها) مما حال بينهم وبين تعيين النشاط المضاد للفيروس. أما باقي المستخلصات فلها نشاط مضاد لفيروس السعار بدرجات متفاوتة ما عدا النعناع و الفلفل الأسمر رغم عدم وجود تأثير سام لهم. هذه النتائج تدل على احتواء المستخلصات الخام على كثير من المواد المضادة للأكسدة والمضادة للنشاط الفيروسي وتوضح ما للمستخلصات الزيتية من تأثيرات سامة على الخلايا علاوة على تفاوت النشاط المضاد للأكسدة والمضاد الفيروسي وعدم وجود علاقة وثيقة بينهم، مما جعلنا نهتم بالمحتوى البيولوجي لهذه الأعشاب والتوابل شائعة الاستخدام حيث أنها قد تكون مصدر لعلاج العديد من الأمراض الفيروسية.