Journal of Medicinal Plants and By-products (2013) 1: 17-22

Original Article

Effect of Drought Stress on Chemical Constituents, Photosynthesis and Antioxidant Properties of *Rosmarinus officinalis* Essential Oil

Rozbeh Farhoudi[1*]

[1]*Department of Agronomy and Plant Breeding, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran*

Article History: Received: 27 January 2013/Accepted in revised form: 09 April 2013

Abstract

This research was carried out to assess the effect of drought stress on head branch dry weight, photosynthesis, essential oil yield and radical scavenging of rosemary (*Rosmarinus officinalis* L.) in Islamic Azad University, Shoushtar branch, Iran during 2010-2011. The experiments were carried out using complete randomized block design with four replications and drought stress levels included control, medium stress (75% field capacity) and sever stress (55%field capacity). Medium and sever drought stress increased essential oil percentage. Medium drought stress increase rosemary essential oil yield (7.6 g/m^2) compared with control. Sever drought stress decreased essential oil yield (4.1 g/m^2), photosynthesis rate (11.7 µ mol Co^2/cm^2/min) and head branch dry weight (52.7 g/m^2). GC/MS results indicated rosemary major oil components include β-Pinene, 1,8-cineole, ά-bisabololoxide A, α-pinene and ά-bisabolol and drought stress increased these chemical compound compared control. Antioxidant activity was analyzed using the DPPH method. Results indicated that essential oil obtained from medium stress and drought stress exhibited a dose-dependent increase with a radical scavenging effect of 90.0% and 88.0% at 350 µg/ml, compared with BHT (94.0%) at the same concentration. This study showed medium drought stress increased essential oil yield and free radical scavenging capacity in rosemary.

Key words: Rosemary, Drought stress, Lipid peroxidation, Radical scavenging

Introduction

Drought stress is a major problem in Iran. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation [1]. Irrigation water is a limited resource and water-saving practices are highly encouraged. It is therefore important to determine whether drought stress causes physiological consequences in field-grown plants that are persistent and limiting plant growth [2].

Drought stress severely hampered the gas exchange parameters of crop plants and this could be due to decrease in impaired photosynthetic machinery, oxidation of chloroplast lipids and changes in structure of pigments and proteins [1,3,4]. Nogues and Baker [5] found drought stress decreased photosynthesis, plant weight and leaf area in rosemary (*Rosmarinus officinalis*).

Recently, aromatic and medicinal plants have received much attention in food industry. Although, secondary metabolites in medicinal and aromatic plants impressed conventionally by their genotypes and their biosynthesis is strongly influenced by environmental factors too [6]. It means biotic and abiotic environmental factors affect growth parameter, essential oil yield and constituents. Abiotic environmental stresses like drought has the most effects on medicinal plants. Farhoudi [7] found medium drought stress increased essential oil percentage and essential oil yield in chamomile but severe drought stress decreased growth, photosynthesis and essential oil yield in this plant. Khorasaninejad et al. [8] found drought stress decreased growth and essential oil yield of peppermint (*Mentha piperita*) but some of medical compounds increased under drought stress condition.

Oxidative stress makes a cellular damage and lipid peroxidation in biological system and it causes many

*Corresponding author: Department of Agronomy and Plant Breeding, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran.
E-mail Address: rfarhoudi@gmail.com

human diseases, including atherosclerosis, aging and cancer. Thus, increasing antioxidant intake in human diet is an important way to minimize such oxidative damages. Therefore, researches concerning medical plant essential oils as potential antioxidants for treatment of human diseases, prevention and treatment of free radical-related disorders, and preserving foods are important. Hernández-Ceruelos et al. [9] reported chamomile essential oil is an efficient chemo protective agent against damage induced by daunorubicin in precursor cells of the germinal line of mice, and therfore its antioxidant capacity may induce this effect.

Rosemary (*Rosmarinus officinalis* L.), is a member of the Lamiaceae family, it is widely cultivated all over the world as an ornamental and aromatic plant. This plant is used in traditional medicine and pharmaceutical products as astringent, tonic and digestive [10]. Moreno et al. [11] found rosemary had phenolic compounds with antimicrobial activity. Kadri et al. [12] reported rosemary leaves had antioxidant effect and reactive oxygen radical scavenging activity. They found Major components in rosemary oil are camphor, α-pinene, β-pinene, 1,8-cineole, bornyl acetate and borneol. Rosemary is known to be an anti- inflammatory, anti-spasmodic and anti-bacterial. Our objective in this study was to study and identify the effect of drought on photosynthesis, chemical and to evaluate rosemary antioxidant activity by using DPPH assay.

Material and Methods

The experiments were carried out using complete randomized block design with four replications at the experimental field, Islamic Azad University, Shoushtar branch, Iran, during 2010-2011. Plants density was 10 plants per m^2 in each plot. Rosemary slips were sown at nursery in March 2010. Slips transferred to field at September 2010. Irrigation treatments started in drought season (April – October). Drought stress levels included control (90% field capacity), medium stress (75% field capacity) and sever stress (55%field capacity).

Head branch harvested in the end of July 2011. Head branch dry weight calculated as 1 m^2 in each plot. Soon after each harvest head branches dried at room temperature (22- 25 °C) for 2 weeks for essential oil assay.

Chlorophyll a and b contents were measured by Gross [13] method. For photosynthesis assay, an infrared open gas exchange system LI-6400 was used to measure photosynthesis on the same leaf in all plants.

Lipid peroxidation assay was done by Valentovic et al. [14] method. 0.1 g of rosemary leaves was mixed with 1.5 ml of 10% trichloroacetic acid (TCA) and centrifuged at 10,000×g for 15 min, and 350 µl of supernatant was mixed with 0.6% (w/v) thiobarbituric acid. The resulting mixture was heated at 95 °C for 30 min and then quickly cooled on ice for 5 min. After centrifugation at 10,000×g for 10 min at 4 °C, the absorbance of the reaction mixture was measured at 450, 532 and 600 nm. The MDA concentration (µM) was calculated according to the formula below:

$$MDA = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

Where A_{532}, A_{600}, and A_{450} represent the absorbance of the mixture at 450, 532, and 600 nm, respectively.

Clevenger apparatus was used to extract oils by hydro distillation of rosemary leaves for 3 h according to the method described in British Pharmacopeia [15]. The rosemary essential oil analyzed by GC/MS (6890 N network GC system and 5975 B intent MSD). Carrier gas was helium with flow rate of 1.7 ml/min. the initial temperature of column was 50 ºC then heated to 250 ºC and constant for 20 min. the identification of each component was studied by mass spectral data [16].

DPPH Radical Scavenging Assay

Rosemary essential oil antioxidant activity was analyzed using the 1,1-diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging according to the method employed by Bersuder et al. [17] Samples containing DPPH and TiO_2 were made up to 3 ml with methanol and placed in a 10 mm quartz cuvette. The samples were kept in the dark and the absorbance at 517 nm measured every 5 minutes. The samples required mixing before each measurement was taken in order to re-disperse the TiO_2. The DPPH radical-scavenging activity is calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{blank} - A_{sample})/A_{blank}] \times 100.$$

Where A_{blank} and A_{sample} are the absorbance of the control (blank) and the sample, respectively.

One way ANOVA was carried out using MSTATC software. Post hoc tests (Duncan) were performed only if F-test was significant at $p \leq 0.01$.

Results

Drought stress showed notably reduced head branch dry weight and photosynthesis rate. Under normal conditions (I 90) and medium drought stress

condition (I 75) head branch dry weight were higher than sever drought stress (I 55) treatment (Table 1). Severe drought stress decreased head branch dry weight to 52.7 g/m^2.

In this study, leaf photosynthesis was affected by drought stress was quite significant. The lowest leaf photosynthesis rate (11.7 μ mol Co^2/cm^2/min) obtained of severe drought stress. Drought stress reduction chlorophyll a and chlorophyll b content in rosemary leaf (Table 1). The minimum amount of chlorophyll a (0.61 mg/g) and chlorophyll b (0.76 mg/g) were obtained from sever drought stress (I 55) treatment. Medium drought stress (I 75) significantly decreased chlorophyll a content compared with control but chlorophyll b content did not change comparing with control, in this condition (Table 1).

Results indicated drought stress increased lipid peroxidation in rosemary leaves and maximum Malondialdehyde (MDA) concentration (10.8 nmol/g FW) obtained from I55 treatment (Table 1).

Results showed sever drought stress (I 55) decreased rosemary head branch essential oil yield (4.1 g/m^2) compared with control (5.7 g/m^2) but Medium drought stress (I 75) significantly increased rosemary head branch essential oil yield (7.6 gr/m^2) compared with control and sever drought stress (Table 1). Both drought stress levels increased rosemary head branch essential oil percentage comparing with control (Table 1). However, total production of essential oil is controlled not only by essential oil percentage but also by dried weight yield, which as our study shows that it was significantly affectedby drought stress.

Gas-chromatographic analysis of the composition of rosemary essential oil under drought stress condition showed in table 2. In control condition rosemary major compounds were 1,8-cineole (18.3%), α-pinene (11.2%), Borneol (8.1%), Camphene (6.6%) and sabinene (5.2%). In medium drought stress (I75) major compounds were 1,8-cineole (20.2%), α-pinene (13.4%), terpinene-4-ol (10.4%), β-pinene (7.2%) and ά –bisabololoxide (5.6%). In sever drought stress (I55) essential oil characterized by 1,8-cineole (22.0%), α-pinene (13.9%), β-pinene (7.7%), ά-bisabolol (7.1%) and ά-bisabololoxide A (5.8%). Results indicated that drought stress increased some major oil components include β-pinene, ά-bisabolol, α-pinene, 1,8-cineole and ά -bisabololoxide A.

Results also indicated that essential oil obtained from rosemary under drought stress exhibited a dose-dependent increase with a radical scavenging effect of 90.0% (I 75) and 88.0% (I 55) at 350 μg/ml, which is close to the DPPH inhibition of the positive control BHT (94.0%) at the same concentration. essential oil obtained of control treatment exhibited a dose-dependent increase with a radical scavenging effect of 76.0% at 350 μg/ml and shown that the rosemary essential oil at control condition exhibited the weakest antioxidant effects than BHT or other essential oils at drought stress conditions (Fig. 1). IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Comparing the DPPH scavenging activity of I 75 (102.1 μg/ml) and I 55 (98.9 μg/ml) and those expressed by BHT (100.0 μg/ml), it was shown that the essential oil of rosemary under drought stress condition exhibited the good antioxidant effects compared with control (Fig. 1).

Discussion

Results showed that severe drought stress (I 55) decreased head branch dry weight, essential oil yield and leaf photosynthesis in rosemary compared with medium drought stress (I 55) and control (I 90) but sever drought stress increased lipid peroxidation in compared with other treatments. The reduction in plant growth may be due to disturbance in photosynthesis under stress condition and suppression of the plant growth Table1. Baghalian et al. [18] and Farhoudi [7] reported drought stress decreased shoot weight, flower yield and shoot height of chamomile. Drought stress reduced reduction photosynthesis rate, chlorophyll a and chlorophyll b content in rosemary (Table 1).

Table 1 Means comparison effect of drought stress on rosemary growth and essential oil yield

Drought stress level	Essential oil percentage	Essential oil yield (g/m^2)	Head branch dry weight (g/m^2)	Chlorophyll b (mg/g FW)	Chlorophyll a (mg/g FW)	photosynthesis rate μ mol Co^2/cm^2/min)	MDA concentration (nmol/g FW)
Control (I90)	0.73 ^{b}	5.4 ^{b}	88.0 ^{a}	0.93 ^{a}	1.02 ^{a}	18.3 ^{a}	3.15 ^{b}
Medium (I75)	0.84 ^{a}	7.6 ^{a}	79.1 ^{a}	0.89 ^{a}	0.79 ^{b}	16.9 ^{a}	4.32 ^{b}
Sever (I55)	0.87 ^{a}	4.1 ^{c}	52.7 ^{b}	0.76 ^{b}	0.61 ^{c}	11.7 ^{b}	10.8 ^{a}

Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan

Table 2 Chemical composition and percentage composition of the rosemary essential oil

Control (I90)		Medium stress (I75)		Sever stress (I55)	
Compound	%	Compound	%	Compound	%
1,8-cineole	18.3	1,8-cineole	20.2	1,8-cineole	22.0
α-pinene	11.2	α-pinene	13.4	α-pinene	13.9
Borneol	8.1	terpinene-4-ol	10.8	β-pinene	7.7
Camphene	6.1	β-pinene	7.2	ά-bisabolol	7.1
Sabinene	5.2	ά-bisabololoxide	5.6	ά -bisabololoxide A	5.8
β-pinene	4.9	ά-bisabolol	4.9	a-bisabololoxide	5.7
bisabololoxide A	4.8	Camphene	4.4	Camphor	4.3
a-bisabololoxide	4.6	a-bisabololoxide A	4.4	Piperitone	3.3
ά-bisabolol	4.4	γ-eudesmol acetate	3.1	α-copaene	3.0
Chamazulene	4.3	*trans*-b-farnesene	3.1	Sabinene	2.2
terpinene-4-ol	3.1	Chamazulene A	2.3	γ-eudesmol acetate	2.1
Δ3-carene	2.4	Sabinene	2.1	Terpinene-4-ol	2.1
γ-eudesmol acetate	1.6	α-cadinene	1.6	β-myrcene	2.0
α-copaene	1.1	Germacrene-D	1.6	Chamazulene	2.0
α-cadinene	0.93	Borneol	1.4	Azulene	1.9
cis-β-ocimene	0.88	Azulen	0.93	Borneol	1.8
Germacrene-D	0.66	Δ3-carene	0.91	α-tricyclene	1.8
Azulen	0.62	α-humulene	0.51	Thujanol	1.4
Tricyclene	0.43	Thujanol	0.46	Germacrene-D	1.0
Chrysanthenone	0.43	α-tricyclene	0.31	α-cadinene	0.36
β-myrcene	0.31	Chrysanthenone	0.29	Chrysanthenone	0.31
α-humulene	0.26	α-phellandrene	0.25	α-terpineol	0.22
α-terpineol	0.22	Pentadecanoic acid	0.21	Dodecane	0.22
Pentadecanoic acid	0.17	*trans*-caryophyllene	0.19	Pentadecanoic acid	0.20
α-phellandrene	0.17	*trans*-β-ocimene	0.19	*trans*-β-ocimene	0.21
Dodecane	0.12	Dodecane	0.18	α-Humulene	0.11
Total	91.4		95.9		96.7

The minimum amount of photosynthesis rate (11.7 μ mol $Co^2/cm^2/min$), chlorophyll a (0.61 mg/g) and chlorophyll b (0.76 mg/g) were obtained from I 55 treatment.

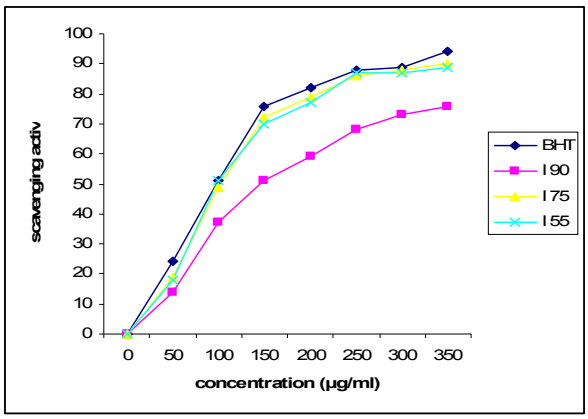

Fig. 1 Free radical scavenging activity of rosemary essential oil under drought stress condition

Pirzad et al. [4] have associated the increased electrolyte leakage to reductions in chlorophyll concentrations. Results indicated drought stress increase lipid peroxidation in rosemary leaf (Table 1).Lipid peroxidation and MDA increased due to disturbed chlorophyll and cell membrane stability in plant cells. Farhoudi [7] found drought stress increased MDA concentration in German chamomile but decreased total chlorophyll content and photosynthesis rate. Water stress decreased plant photosynthesis, chloroplast content and cell membrane stability in plants [2].

Rosemary major compounds were camphene, β-Pinene, ά–bisabololoxide, ά-bisabolol, α-pinene, 1, 8-Cineole and sabinene (Table 2). Kadri et al. [12] and Tavassoli et al. [10] found that 1,8-cineole, camphene, β-Pinene, trans-caryophyllene, borneol, camphor, α-pinene and α-thujone were major compounds in rosemary essential oil. Results indicated that some major compounds like β-pinene, 1,8-cineole, α-pinene, ά-bisabolol and ά-bisabololoxide A percentage in rosemary essential oil increased under drought stress condition [18,19]. Genetic and environmental conditions like drought stress controlled essential oil compounds in medical plants Smelcorevic et al. [21] reported sabinene, α-pinene, 1,8-cineole and borneol are major chemical compounds in *Achillea millefolium* and *Achillea crithmifolia* which have antioxidant capacity.

Essential oil percentage increased in both stress levels but did not show any significant difference

between stress levels (Table 1). Medium drought stress significantly increased head branch essential oil yield (7.6 g/m^2) compared with other treatments (Table 1). Shabih et al. [22] reported when water stress does not decrease plant growth, the production of secondary metabolites such as essential oil is even stimulated by limited stressful environment conditions. In our study, rosemary essential oil yield was affected by drought stress because drought stress decreased rosemary head branch. Razmjoo et al. [23] observed drought stress decreased the number of branches, plant height and flowers essential oil content of chamomile and Khorasaninejad et al. [8] reported drought stress decreased essential oil percentage and essential oil yield in peppermint. Severe drought stress increased rosemary essential oil but decreased head branch dry weight and it made loss in rosemary essential oil yield. The reduction in essential oil content may be due to disturbance in photosynthesis and carbohydrate production under stress condition and suppression of the plant growth.

The DPPH method has been widely applied for estimating antioxidant activity of various natural products in recent years. This method has the advantages of being a stable, easy and rapid way to study the antioxidant activity of food or natural products which act as free radical- scavengers or hydrogen donors *in vitro*. The screening of the potential activity of essential oil may require a combination of different methods to describe the antioxidant properties of the sample in more details. under drought stress our results indicated some rosemary essential oil compounds like 1,8-cineole, ά-bisabolol, α-pinene and β-pinene which were higher compared with control condition (Table 2). Results in Fig. 1 showed the DPPH scavenging ability of rosemary under drought stress condition can be attributed to the presence of some components that have antioxidant activity, for example: β- pinene, 1,8 cineole, α-pinene, [24], ά-bisabolol and ά - bisabololoxide A [18].

This study indicated that drought stress increased antioxidant activity of rosemary. In conclusion, results showed that rosemary can be grown successfully on middle drought stress because rosemary essential oil yield and antioxidant medicinal compounds like 1,8-cineole, ά-bisabolol, α-pinene and β-pinene percentage increased under medium drought stress condition (I 75) but severe drought stress treatments (I 55) significantly decreased rosemary growth, photosynthesis rate and essential oil yield.

References

1. Abdul Jaleel C, Manivannan P, Wahid A, Farooq M, AL-Juburi H J, Somasund R, Panneersel R. Drought stress in plants: A review on morphological characteristics and pigments composition. Int J Agric Biol. 2009;11:100-105.
2. Anjum S H, Xie X, Wang L C, Farrukh Saleem M, Man C, Lei W. Morphological, physiological and biochemical responses of plants to drought stress. Afr J Agric Res. 2011;6:2026-2032.
3. Nakayama N, Saneokai H, Moghaieb R E A, Premachander G S, Fujita K. Response of growth, photosynthetic gas exchange, translocation of 13C-labelled photosynthate and N accumulation in two soybean (*Glycine max* l. merrill) cultivars to drought stress. Int J Agric Biol. 2007;9:669-674.
4. Pirzad A, Shakiba M R S, Zehtab-Salmasi A, Mohammadi R, Darvishzadeh S, Samadi S. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla*. J Med Plants Res. 2011;5:2483-2488.
5. Nogues S, Baker N R. Effect of drought on photosynthesis in Mediterranean plants grown under enhance UV-B radiation. J Exp Bot. 2000;51:1309-1317.
6. Yazdani D, Jamshidi H, Mojab F. Compare of essential oil yield and menthol existent in Peppermint (*Mentha piperita* L.) planted in different origin of Iran. J Med Plants. 2002;3:73-78.
7. Farhoudi R. Effect of drought stress on growth and essential oil yield of German chamomile. Res Crop. 2012;14:123-129.
8. Khorasaninejad S, Mousavi A, Soltanloo H, Hemmati K, Khalighi A. The effect of drought stress on growth parameters, essential oil yield and constituent of peppermint. J Med Plants Res. 2011;5: 5360-5365.
9. Hernández-Ceruelos A, Madrigal-Santillán E, Morales-González J A, Chamorro-Cevallos G, Cassani-Galindo M, Madrigal-Bujaidar E. Antigenotoxic effect of *Chamomilla recutita* essential oil in mouse spermatogonial cells and determination of its antioxidant capacity *in vitro*. Int J Mol Sci. 2010;11:3793-3802.
10. Tavassoli S K, Mousavi S M Z, Emam-Djomeh A, Razavi S H. Chemical composition and evaluation of antimicrobial properties of *Rosmarinus officinalis* L. essential oil. Afr J Biotechnol. 2011;10:13895-13899.
11. Moreno S, Scheyer T, Romano C S, Vojnov A A. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Res. 2006;40:223-231.
12. Kadri A, Zarai Z, Ben Chobba I, Békir A, N. Gharsallah M, Damak R, Gdoura R. Chemical constituents and antioxidant properties of Rosmarinus officinalis L. essential oil cultivated from South-Western Tunisia. J Med Plants Res. 2010;5:5999-6004.
13. Gross J. Pigment in vegetables: chlorophylls and carotenoids. Van Nostrand Reinhold, New York, 1991.
14. Valentovic P, Luxova M, Kolarovi L, Gasparikora O. Effect of osmotic stress on compatible solutes content, memberane stability and water relation in two maize cultivars. Plant Soil Environ. 2006;52:186-191.

15. British Pharmacopoeia commission. British Approved Names. A dictionary of Drug Names for Regulatory use in the UK, second ed. The Stationary Office Publisher .2002.
16. Mazandarani M, Majidi Z, Zarghami- Moghadam P, Abrodi M, Hemati H, Fathiazad F. Essential oil composition, total flavonoid, anthocyanin and antioxidant activity in different parts of *Artemisia annua* in two location (north of Iran). J Med Plants By-prod. 2012;1:13-21.
17. Bersuder P, Hole M, Smith,G. Antioxidants from a heated histidineglucose model system I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high performance liquid chromatography. J Am Oil Chem Soc. 1998;75:181-187.
18. Baghalian K, Abdoshah S, Khalighi-Sigaroodi F, Paknejad F. Physiological and phytochemical response to drought stress of German chamomile (*Matricaria recutita* L.). Plant Physiol Biochem. 2011;49:201-207.
19. Bua-In S , Paisooksantivatana Y. Essential oil and antioxidant activity of Cassumunar ginger (Zingiberaceae: *Zingiber montanum*) collected from various parts of Thailand. Kasetsart J. 2009;43:467 – 475.
20. Baghalian K, Haghiry A, Naghavi M R, Mohammadi A. Effect of saline irrigation water on agronomical and phytochemical characters of chamomile (*Matricaria recutita* L.). Scientia Hort. 2008;116:437–441.
21. Smelcorevic A, Lamshoeft M, Radulovic N, Ilic D, Palic R. LC/MS analysis of the essential oils of *Achillea millefolium* and *Achillea crithmifolia*. Chromatographia 2010;71:113-116.
22. Shabih F, Farooqi A H A, Ansari S R and Sharma S. Effect of water stress on growth and essential oil metabolism in *Cymbopogon martinii* cultivars. J. Essent Oil Res. 1999;11:491-496.
23. Razmjoo K, Heydarizadeh P, Sabzalian M R. Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomilla*. Int J Agric Biol. 2008;10:451-454.
24. Houghton P J. Activity and constituents of Sage relevant to the potential treatment of symptoms of Alzheimer's disease. Herbal Gram. 2004;61:38-54.