Potential Beneficial Effect Of Functional Food Components In Alzheimer' Disease

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ABSTRACT: Elevated oxidative stress, inflammation and reduced acetylcholine have been reported in Alzheimer' disease. The present study has been postulated in a trial to find out functional food components that may reduce the deterioration or retard the onset of the disease through ameliorating the aforementioned criteria. The tested fractions were methanol and petroleum ether extracts of Carica fruit, leaves and seed, Origanum herb, ginger, grape leaves and fruit (raisin), and fig fruit. Antioxidant effect and cholinesterase inhibiting activity have been tested in-vitro. Anti-inflammatory effect was evaluated in carragenan model in rats. Total phenolic contents of the different tested plants were determined. G.C. analysis of unsaponifiable matter of the ginger lipid fraction was carried out. Results showed that the highest antioxidant activity belonged to methanol extract of ginger (88%), Carica leaves (85%) and Origanum (74%) while the most potent anti-inflammatory effect was attributed to methanol extract of Carica fruit followed by the methanol extract of grape leaves then methanol extract of fig. Petroleum ether extract of ginger showed the highest cholinesterase inhibiting activity (85%) followed by petroleum ether extract of Carica seeds (73%) then methanol extract of raisin (66%), petroleum ether extract of Carica leaf (65%) and petroleum ether extract of fig (63%). Results of total phenolic showed grape leaves to contain the highest content (93.52 mg gallic acid equivalent/g dry sample) followed by Origanum (77.93), Carica leaves (41.1), Carica fruit (30.29), ginger (29.29), Carica seed (26.70), and raisin (23.75). Figs showed the least phenolic content (14.27). G.C. analysis of unsaponifiable lipid fraction of ginger showed total identified phytosterol to be 0.738% and total hydrocarbon to be 86.242% of total unsaponifiable matter. **Conclusion:** The highest antioxidant and cholinesterase inhibiting activity were attributed to methanol and petroleum ether extract of ginger respectively. While methanol extract of Carica fruit was superior as anti-inflammatory agent. Combination of the previously mentioned extracts may have potential beneficial effect towards Alzheimer disease. . [Academia Arena, 2009;1(2):55-68]. ISSN 1553-992X

Keywords: Beneficial Effect; Functional Food Components; Alzheimer' Disease

INTRODUCTION

Alzheimer' disease (AD) is a neuropsychiatric condition with progressive neurodegeneration, dementia and decline of cognitive function, usually accompanied by behavioral disturbances [1]. AD is the fourth cause of death in Europe and U.S.A. [2]. It appears as a new epidemic threatening of human civilization in the next century. Its incidence, at present, doubles every five years. AD affects almost 4 million Americans (1.5% of the population) and costs $ 65 billion annually [3]**.** In Egypt, no actual survey showed the prevalence rate of Alzheimer; however a study in Assiut governorate showed 2.2% of populations over the age of 60 have Alzheimer [4]**.**

Previous studies demonstrated elevated oxidative stress in brains and peripheral tissues in AD patients, as well as in animal models of AD [5, 6]**.** AD is characterized by senile plaques, fibrillary tangles and a reduction of cholinergic neurons in brain. The major component of senile plaques was amyloid beta peptide (Aβ), a 39 – 43 amino acid peptide. Aβ was shown to have the potential to induce oxidative stress and inflammation in the brain, which were postulated to play important roles in the pathogenesis of AD [7]**.** Aβ induces the production of hydrogen peroxide and lipid peroxide in neurons [8]**.** In addition, Aβ has been reported to induce superoxide [9] and pro-inflammatory cytokines such as interleukin-1β (IL-1β) [10] in brain. Increased IL-1β in brain may contribute to memory deficit [11]**.** There is a strong evidence for the presence of a localized inflammatory reaction in AD brains that may be involved in neurodegeneration in

AD [12]. Levels of tumor necrosis factor, an inflammatory marker, were elevated in CSF and serum [13]. During the past two decades, a wide range of inflammatory markers, typically absent in the normal elderly population have been reported in AD brains [12] and accumulating evidence suggested that sustained brain inflammation might be an essential cofactor in AD pathogenesis [14]. On the other hand, there is evidence suggesting that oxidative stress contributes to the formation of amyloid plaques and neurofibrillary tangles [15].

Brain levels of cholesterol and choline which are precursor of the neurotransmitter acetylcholine were shown to be reduced in AD [16]. Also an increased activity of acetylcholinesterase has been reported in AD [17]. Both previously mentioned changes may result in reduced acetylcholine level which may contribute to dementia.

Acetylcholinesterase inhibitors, which enhance cholinergic transmission by reducing the enzymatic degradation of acetylcholine, are actually the most important way for reducing the risk of AD [18] together with antioxidant and anti-inflammatory agents may have potential beneficial effects towards AD.

The aim of the present study is to evaluate in-vitro antioxidant and cholinesterase inhibiting activity and in-vivo anti-inflammatory effect of different fractions of selected plant food. The promissing fractions may have collectively potential beneficial effect as functional food components towards AD. The aim included determination of total phenolic content of the studied plants and the possible active constituents of lipid compartment of the most efficient fraction.

MATERIALS AND METHODS

Materials

Plant materials

The plant materials used in this study were the leaves, seeds and fruits of Carica papaya L. family Caricaceae (papaya), the herb of Origanum majorana L. family Lamiaceae (marjoram), leaves and fruits (seedless) of Vitis vinifera L. family Vitaceae (grape), fruits of Ficus carica L. family Moraceae (fig) and the rhizome of Zingiber officinale family Zingiberaceae (ginger). All plant materials were purchased from local market in Giza city, Egypt except for papaya which was obtained from Ministry of Agriculture, Egypt.

Animals

Female albino rats of sprague-Dawley strain of 109 g ± 1.455 Mean ± SE body weight were used in acute inflammation test and were maintained on laboratory stock diet. Animals were kept individually in stainless steel cages at room temperature of about 25 ±2 ºC, food and water were given ad-libitum. Rats were supplied by the animal house of the National Research Centre, Egypt.

Major chemicals

- D,L α-tocopherol, β-carotene, linoleic acid, tween 20 and Folin-Ciocalteu reagent, all were purchased from Sigma (USA), petroleum ether 40-60ºC and methyl alcohol from BDH Chemical Co, England.
- λ-Carrageenan, Type IV (Sigma, USA): One percent of λ-carrageenan in saline was freshly prepared for induction of acute inflammation
- Acetylthiocholiniodide, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent) was obtained from Fluka Bio-Chemika, Switzerland.

Methods

1- Preparation of Plant Materials

Fruits and leaves of Carica papaya and grape, fig and Origanum majorana herb were all washed by tap water. Carica papaya fruit was peeled and its pulp was cut into small slices, papaya seeds were separated. Also figs were cut into small pieces. All the studied plant parts were dried separately in an air-circulated oven at 40 ºC till complete dryness. All dried plant materials were reduced separately into powder form as far as possible and subjected to successive extraction using soxhlet apparatus.

2- Preparation of Plant Extracts

A known weight of each of the dried plants under study was placed separately in a continuous extraction apparatus (Soxhlet) and subjected to successive extraction using petroleum ether (40-60 ºC) then absolute methanol. Complete extraction has been verified when the extracting solvent became colorless and tested by evaporating small aliquot from it to dryness in a glass watch till no residue was obtained. For each extract the solvent was completely removed by distillation under reduced pressure using rotary evaporator and dried to a constant weight in a vacuum dessicator over anhydrous calcium chloride.

3- Preparation of the Extracts' Doses for Acute Inflammation Test

The dry methanolic extracts of the studied plants were dissolved separately in distilled water, whereas the dry petroleum ether extracts were suspended in distilled water using gum acacia. Only one dose (500 mg/kg rat body weight) of each plant extract was used in the acute inflammation test.

In-vitro antioxidant activity using β-carotene bleaching method [19]

Antioxidant activity of the different extracts was determined according to the β-carotene bleaching method using D-L-α-tocopherol as standard. One ml of β-carotene solution (0.2 mg/ml chloroform) was transferred to different round-bottom flasks (100 ml) containing 0.02 ml linoleic acid and 0.2 ml tween 20. Each mixture was then dosed with 0.2 ml of 80 % MeOH (as control) or α-tocopherol (50 mg/L) (as standard) or the plant extracts. After evaporation to dryness under vacuum at room temperature, 50 ml of oxygenated distilled water was added to each flask and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autoxidation at 50˚C for 2h. The absorbance of the samples at 470 nm was measured immediately after their preparation before thermal auto oxidation (t = 0 min) and at the end of the experiment (t = 120 min) using UVPC spectrophotometer. All samples were assayed in duplicate and the mean was calculated. Antioxidant activity (AA) was calculated as percent inhibition of oxidation relative to control sample, using the following equation [20]

$$AA = \frac{R_{control} - R_{sample\ or\ standard}}{R_{control}} \times 100$$

where $R_{control}$ **and** $R_{sample\ or\ standard}$ were the bleaching rates of *β*-carotene in reactant mixture of the control and sample or standard, respectively.

In- vitro acetylcholinesterase inhibiting activity

The purpose of this assay is to screen plants' extracts for inhibition of Acetylcholinesterase activity. Inhibitors of this enzyme may be useful for the treatment of AD. Acetylcholinesterase inhibiting activity was determined according to the method of Vogel and Vogel [21] which is a modification of Ellman [22] procedure.

Tissue preparation

Male albino rats were decapitated, brains were rapidly removed, corpora striata dissected free, weighed and homogenized in 19 volumes of 0.05 M phosphate buffer pH 7.2. A 25 µl aliquot of this suspension is added to 1 ml of different plants' extracts or the vehicle and re-incubated for 10 min at 37 ºC.

Enzyme activity was measured at 412 nm using the UVPC spectrophotometer. Blank values were determined for each run to control for non-enzymatic hydrolysis of substrate and these values were subtracted from that of all samples and control. The change in absorbance for 6 min for control and samples were read and used for calculating the inhibiting activity, through plotting the absorbance against time and the slope was calculated.

Calculation

$$\% \text{ Inhibition} = \frac{\text{Slope of the control} - \text{slope of the plant extract}}{\text{Slope of the control}} \times 100$$

In-vivo anti-inflammatory activity using carrageenan model of acute inflammation

Evaluation of the anti–inflammatory activity of different plant extracts under investigation was carried out using carrageenan model of acute inflammation

- Rats maintained on laboratory stock diet were fasted for 16 hrs before starting the experiment and divided into 17 groups, each comprised six rats.

The groups were:

- Two control groups where rats received no plant extract but only given the vehicle.
- Test groups, 15 groups where rats of the different groups were given one oral dose (500 mg/kg rat body weight) of either methanol or petroleum extract of Origanum majorana, Carica papaya (seed, fruit and leaves), fig, ginger and grape (fruits and leaves).
- After an hour of the oral administration of extract or the vehicle, all rats of test and control groups were injected into the sub-planter region of the right hind paw (foot) with 1% λ- carrageenan suspension (0.05 ml / animal) [23]**.**
- Paw thickness was measured using vernier calipers immediately before the injection of carrageenan (Zero time) and after 30 min, 1, 1.5, 2, 3 and 4 hours of carrageenan injection.
- The thickness of inflammation was calculated by subtracting paw thickness at zero time from paw thickness at different time intervals for each rat.
- The mean thickness of inflammation of the hind paw of rats given different plant extracts was compared with that of the control inflamed rats by applying the statistical analysis of the t-student's test.

Extraction and determination of total phenolic content (TPC)

TPC was extracted from the dry powder samples of the plant foods under study according to the method of **Velioglu *et al.* [19]**. Each sample (200 mg.) was extracted separately with 2 ml of methanol (80%) containing 1% HCl at room temperature in a shaker for 2 hours. Then, centrifuged at 3000 r.p.m for 10 min. The upper layer was collected in different clean tubes and re-extraction of the residue was carried out using the same previous procedure. The second extract was added to the first and used for determination of TPC. Total phenolics were determined colorimetrically in the extracted samples using Folin-Ciocalteu reagent [24]**.** The reaction mixture contained 200 ul of extracted samples, 1000 ul of freshly prepared diluted Folin-Ciocalteu reagent and 800 ul of sodium carbonate solution (7.5%) were mixed and kept in the dark at room temperature for 30 min to complete the reaction. Absorbance was measured at 765 nm using UVPC spectrophotometer. Gallic acid was used as a standard and results were calculated as mg gallic acid equivalent per gm of dry sample. The reaction was conducted in triplicate and results were averaged.

Determination of the active constituents in the unsaponifiable matter of lipid fraction of ginger [25].

Five grams of lipid fraction of ginger was saponified by refluxing with 10% alcoholic potassium hydroxide. After dilution with distilled water, the unsaponifiable fraction was extracted with ether. Both aqueous (saponifiable) and nonaqueous portions (unsaponifiable) were separated in separating funnel. The ether was evaporated, and the extract was weighed and analysed by G.L.C. GLC conditions were; column: 10% OV-101 packed column; stationary phase: chromosorb WHP; detector temperature: 290ºC; injector temperature, 28ºC; carrier gas N_2, flow-rate 30 ml/min; air flow-rate: 300 ml/min; H_2, flow-rate 30 ml/min; detector FID; chart speed: 0.5 cm/min; oven program: initial temperature, 70ºC; final temperature, 270ºC, total time, 85 min. Identification of hydrocarbons and sterols contents of the unsaponifiable fraction was carried out by comparison of their retention times with the co-injected reference phytosterols and hydrocarbons. Quantification was based on peak area integration.

RESULTS

The antioxidant activity of different extracts tested in the present study are shown in Table (1). It can be noted that the highest antioxidant activity belonged to methanol extract of ginger (88%), Carica leaves (85%) and Origanum (74%). Petroleum ether extract of ginger and Carica leaves were more or less of the same antioxidant activity (72% and 73% respectively). Methanol extract of grape leaves and fruit (raisin) were 65% and 66% respectively. The antioxidant activity of petroleum ether extract of grape leaves and Origanum was 64% and 59% respectively. Petroleum ether extract of Carica fruit showed 53% antioxidant activity. The least antioxidant activity was attributed to petroleum ether extract of Carica seed, methanol extract of Carica fruit, Carica seed and fig and petroleum ether extract of fig (50%, 40%, 37%, 38% and 26% respectively).

Table (1): Antioxidant activity of different plant extracts

Sample	Antioxidant activity %(AA %)
Methanol extract of ginger	88
Methanol extract of Carica papaya leaves	85
Methanol extract of Origanum majorana	74
Petroleum ether extract of Carica papaya leaves	73
Petroleum ether extract of ginger	72
Methanol extract of grape fruit (raisin)	66
Methanol extract of grape leaves	65
Petroleum ether extract of grape leaves	64
Petroleum ether extract of Origanum majorana	59
Petroleum ether extract of Carica papaya fruit	53
Petroleum ether extract of Carica papaya seed	50
Methanol extract of Carica papaya fruit	40
Methanol extract of fig	38
Methanol extract of Carica papaya seed	37
Petroleum ether extract of fig	26
Standard (D,L α- tocopherol (50mg/L)	92

Table (2): Acetylcholinesterase inhibiting activity

Plant extract	% Inhibition
Petroleum ether extract of ginger	85
Petroleum ether extract of Carica seeds	73
Methanol extract of grape fruits	66
Petroleum ether extract of Carica leaves	65
Petroleum ether extract of fig	64
Methanol extract of ginger	49
Methanol extract of Carica fruit	49
Methanol extract of fig	45
Methanol extract of Origanum	44
Methanol extract of grape leaves	32
Methanol extract of Carica seeds	32
Petroleum ether extract of grape leaves	30
Petroleum ether extract of Origanum	23
Methanol extract of Carica leaves	12
Petroleum ether extract of Carica fruit	4

The acetylcholinesterase inhibiting activity of different plant extracts are shown in Table (2). Petroleum ether extract of ginger, petroleum ether extract of Carica seed and methanol extract of grape fruit produced the highest inhibition of acetylcholinesterase activity (85%, 73% and 66% respectively). Petroleum ether extract of Carica leaves and fig have nearly the same effect (65% and 64% respectively). Also it was shown that both, methanol extract of ginger and Carica fruit have equal effect (49%). Methanol extract of fig and Origanum were more or less of the same effect (45% and 44% respectively). Whereas methanol extract of grape leaves and Carica seeds were shown to have similar effect (32%). Finally, petroleum ether extract of grape leaves, Origanum, Carica fruit, and methanol extract of Carica leaves showed the least anti-cholinesterase activity (30%, 23%, 4% and 12% respectively).

The results of acute inflammation are present in Tables (3, 4). In table 3, mean hind paw thickness of rats of the different experimental groups can be seen, table 4 showed the calculated inflammation thickness at different time intervals.

In the present study different plant extracts were tested in carrageenan model in rats. It was noticed that methanol extract of Carica fruit showed the most potent anti-inflammatory activity; its significant anti-inflammatory effect started 0.5 hr after carrageenan injection (40%) and increase gradually till reach its maximum activity (68%) after 4 hrs from injection. Methanol extract of grape leaves and figs followed methanol extract of Carica fruit in potency as anti-inflammatory agent. The anti-inflammatory activity profile of both methanol extract of grape leaves and petroleum ether extract of Carica leaves was similar where both showed significant activity after 0.5 hr from carrageenan injection and reach their maximum activity after 1 hr (61% and 52% respectively). The activity started to decline 1.5 hr after carrageenan injection till 4 hrs in case of grape leaves whereas on Carica leaves extract administration it showed slight increase again after 4 hrs. Both methanol extract of grape fruit and Carica seed exhibited similar activity profile as their significant anti-inflammatory activity started one hr after carrageenan injection and continued increasing until the 4^{th} hr. Methanol and petroleum ether extract of ginger exhibited similar activity profile in that both started their significant activity 1.5 hr after carrageenan injection and increased till reach their maximum activity after 4 hrs (64% and 59% respectively). The methanol extract of fig and Carica leaves in addition to the petroleum ether of fig showed similar anti-inflammatory activity, where their significant activity started 0.5 hr after carrageenan injection and reach their maximum activity 1.5 hr after injection (57%, 51% and 41% respectively), their activity started to decline on the 2^{nd} hr from injection and showed slight increase in activity on the 4^{th} hr but methanol extract of fig possess much more potent activity. The petroleum ether extract of Carica fruit and grape leaves showed weak significant anti-inflammatory activity which started 0.5 hr after carrageenan injection. It is worthy to mention that both methanol and petroleum ether extract of Origanum majorana have the least anti-inflammatory activity (when taking into account the whole studied period) their maximum significant activity was shown on the 3^{rd} hr from carrageenan injection which was equal to 42%. Petroleum ether extract of Carica seed started its significant activity 1.5 hr after carrageenan injection, its activity showed fluctuation till the 4^{th} hr from injection.

Table (5) showed total phenolic contents of different plant materials as mg gallic acid equivalent per g. dry sample (mg of GAE/g). The highest phenolic contents were attributed to grape leaves followed by Origanum herb then Carica leaves(93.52, 77.93 and 41.1mg of GAE/g of dry weight, respectively). Carica fruit, ginger, Carica seed and grape fruit (raisin) contain medium level of phenolic contents (30.29, 29.29, 26.70 and 23.75 mg of GAE/g of dry weight respectively). It was shown that fig possess the least phenolic content (14.27 mg of GAE/g of dry weight).

The unsaponifiable matter of lipid fraction of ginger was found to be 40%. GLC analysis of such unsaponifiable matter is shown in Table (6). The total identified sterols were 0.738% including campesterol, stigmasterol and beta-sitosterol. Total hydrocarbons were 86.242% of total unsaponifiable matter, the highest percentage was attributed to C15 hydrocarbon (51.938%).

Table (3): Mean hind paw thickness (mm) at different time intervals of carrageenan injection after administration of natural anti-inflammatory agents

GROUPS		TIME						
		Zero	30min	1 hr	1.5hr	2 hr	3 hr	4 hr
Control	Mean	0.3	0.508	0.583	0.608	0.608	0.62	0.642
	±SE	0	0.008	0.028	0.024	0.024	0.021	0.024
MeOH Ext of raisin	Mean	0.3	0.483	0.483	0.442	0.450	0.466	0.450
	±SE	0	0.011	0.017	0.015	0.018	0.017	0.018
MeOH Ext of fig	Mean	0.3	0.467	0.458	0.433	0.450	0.458	0.450
	±SE	0	0.017	0.015	0.017	0.018	0.020	0.029
MeOH Ext of Carica fruit	Mean	0.3	0.425	0.408	0.417	0.408	0.417	0.408
	±SE	0	0.011	0.008	0.011	0.008	0.011	0.027
MeOH Ext of Carica leaves	Mean	0.3	0.450	0.450	0.450	0.458	0.483	0.475
	±SE	0	0.022	0.029	0.029	0.030	0.021	0.031
MeOH Ext of grape leaves	Mean	0.3	0.450	0.408	0.433	0.442	0.466	0.483
	±SE	0	0.018	0.008	0.021	0.020	0.036	0.033
PE Ext of fig	Mean	0.3	0.442	0.466	0.483	0.525	0.542	0.542
	±SE	0	0.015	0.011	0.017	0.011	0.020	0.015
PE Ext of Carica fruit	Mean	0.3	0.458	0.492	0.516	0.525	0.542	0.542
	±SE	0	0.020	0.015	0.021	0.025	0.035	0.024
PE Ext of Carica leaves	Mean	0.3	0.433	0.433	0.483	0.492	0.500	0.500
	±SE	0	0.017	0.025	0.028	0.037	0.039	0.037
PE Ext of grape leaves	Mean	0.3	0.467	0.508	0.516	0.508	0.542	0.566
	±SE	0	0.017	0.015	0.031	0.024	0.037	0.051
Control	Mean	0.3	0.475	0.500	0.542	0.558	0.600	0.625
	±SE	0	0.021	0.022	0.024	0.015	0.026	0.017
MeOH Ext of ginger	Mean	0.3	0.433	0.458	0.442	0.408	0.433	0.416
	±SE	0	0.017	0.008	0.015	0.008	0.021	0.017
PE Ext of ginger	Mean	0.3	0.450	0.442	0.467	0.467	0.425	0.433
	±SE	0	0	0.015	0.011	0.011	0.011	0.017
MeOH Ext of Origanum	Mean	0.3	0.492	0.500	0.510	0.483	0.475	0.492
	±SE	0	0.015	0.018	0.020	0.021	0.021	0.030
PE Ext of Origanum	Mean	0.3	0.475	0.492	0.483	0.525	0.475	0.492
	±SE	0	0.021	0.015	0.011	0.017	0.028	0.027
MeOH Ext of Carica seed	Mean	0.3	0.458	0.442	0.425	0.433	0.442	0.458
	±SE	0	0.015	0.008	0.017	0.021	0.020	0.024
PE Ext of Carica seed	Mean	0.3	0.450	0.467	0.442	0.442	0.475	0.475
	±SE	0	0.018	0.031	0.020	0.015	0.031	0.031

Table (4): The thickness of inflammation of the hind paw (mm) at different time intervals of carrageenan injection after administration of natural anti – inflammatory agents in comparison to control inflamed rats.

GROUPS		TIME					
		30min	1 hr	1.5hr	2 hr	3 hr	4 hr
Control	Mean	0.208	0.280	0.308	0.308	0.317	0.342
	±SE	0.008	0.028	0.024	0.024	0.021	0.024
MeOH Ext of raisin	Mean	0.183	0.183**	0.142****	0.150****	0.167****	0.150****
	±SE	0.011	0.017	0.015	0.018	0.017	0.018
	%inhibition	12	35	54	51	47	56
MeOH Ext of fig	Mean	0.167*	0.158*** *	0.133****	0.150****	0.158****	0.150****
	±SE	0.017	0.015	0.017	0.018	0.020	0.029
	%inhibition	20	44	57	51	50	56
MeOH Ext of Carica fruit	Mean	0.125****	0.108*** *	0.117****	0.108****	0.117****	0.108****
	±SE	0.011	0.008	0.011	0.008	0.011	0.027
	%inhibition	40	61	62	65	63	68
MeOH Ext of Carica leaves	Mean	0.150*	0.150***	0.150****	0.158****	0.183****	0.175****
	±SE	0.022	0.028	0.028	0.030	0.021	0.031
	%inhibition	28	46	51	49	42	49
MeOH Ext of grape leaves	Mean	0.150**	0.108*** *	0.133****	0.142****	0.167****	0.183****
	±SE	0.018	0.008	0.021	0.020	0.036	0.033
	%inhibition	28	61	57	54	47	46
PE Ext of fig	Mean	0.142****	0.167*** *	0.183****	0.225***	0.242*	0.242***
	±SE	0.015	0.011	0.017	0.011	0.020	0.015
	%inhibition	32	41	41	27	24	29
PE Ext of Carica fruit	Mean	0.158*	0.192**	0.217**	0.225*	0.242	0.242**
	±SE	0.020	0.015	0.021	0.025	0.035	0.024
	%inhibition	24	32	30	27	24	29
PE Ext of Carica leaves	Mean	0.133****	0.133*** *	0.183***	0.192**	0.200**	0.200***
	±SE	0.017	0.025	0.028	0.037	0.039	0.037
	%inhibition	36	52	41	38	37	41
PE Ext of grape leaves	Mean	0.167*	0.208*	0.217*	0.208**	0.242	0.267
	±S.E	0.017	0.015	0.031	0.024	0.037	0.051
	%inhibition	20	26	30	33	24	22
Control	Mean	0.175	0.200	0.242	0.258	0.300	0.325
	±SE	0.021	0.022	0.024	0.015	0.026	0.017
MeOH Ext of ginger	Mean	0.133	0.158	0.142***	0.108****	0.133****	0.117****
	±SE	0.017	0.008	0.015	0.008	0.021	0.017
	% inhibition	24	21	41	58	56	64
PE Ext of ginger	Mean	0.150	0.142	0.167**	0.167****	0.125****	0.133****
	±SE	0	0.015	0.011	0.011	0.011	0.017
	% inhibition	14	29	31	35	58	59
MeOH Ext of Origanum	Mean	0.192	0.200	0.208	0.183**	0.175****	0.192****
	±SE	0.015	0.018	0.020	0.021	0.021	0.030
	% inhibition	-10	0	14	29	42	41
PE Ext of	Mean	0.175	0.192	0.183*	0.225	0.175***	0.192****

Origanum	**±SE**	0.021	0.015	0.011	0.017	0.028	0.027
	% inhibition	0	4	24	13	42	41
MeOH Ext of Carica seed	**Mean**	0.158	0.142*	0.125****	0.133****	0.142****	0.158****
	±SE	0.015	0.008	0.017	0.021	0.020	0.024
	% inhibition	10	29	48	48	53	51
PE Ext of Carica seed	**Mean**	0.150	0.167	0.142***	0.142****	0.175**	0.175****
	±SE	0.018	0.031	0.020	0.015	0.031	0.031
	% inhibition	14	17	41	45	42	46

Values significantly different from control: *: p<0.025, **: p<0.010, ***: p<0.005, ****: p<0.001.

Table (5): Total phenolic contents of the different plant materials used in the study.

Plant	Total phenolics as mg gallic acid equivalent/ g dry sample
Grape leaves	93.52
Origanum majorana	77.93
Carica leaves	41.1
Carica fruit	30.29
Ginger	29.29
Carica seed	26.70
Grape fruit	23.75
Fig	14.27

Table (6): Hydrocarbons and phytosterol of unsaponifiable matter of lipid fraction of ginger as percentage of total unsaponifiable.

Hydrocarbons:	%
C11	1.419
C12	0.273
C13	0.250
C14	1.955
C15	51.938
C16	3.617
C17	7.282
C18	1.934
C19	2.184
C20	0.957
C21	3.339
C22	0.973
C23	2.559
C24	2.479
C25	1.636
C26	0.721
C27	1.484
C28	0.792
C29	0.254
C32	0.196
Phytosterols:	0.189
Cholesterol	0.214
Campesterol	
Stigmasterol	0.109
Beta-sitosterol	0.226
Total Identified Hydrocarbons	86.242
Total Identified phytosterols	0.738

DISCUSSION

Increased brain oxidative stress is a key feature of AD and manifests predominantly as lipid peroxidation because of the high content of polyunsaturated fatty acids in central nervous system that are particularly susceptible to oxidation [26]**.** Also the central nervous system is particularly vulnerable to oxidative damage because of its high energy requirement, high oxygen consumption rate, and relative deficit in antioxidant defense system compared with other organs [27]. So antioxidants administration may have beneficial effects in AD [28]**.**

The drugs approved for the AD therapy act by counter acting the acetylcholine deficit, that is, they try to enhance the acetylcholine level in the brain [29]. Acetylcholine is involved in the signal transfer in the synapses. After being delivered in the synapses, acetylcholine is hydrolyzed giving choline and acetyl group in a reaction catalyzed by the enzyme acetylcholinesterase [30]**.** The molecular basis of the Alzheimer drugs used so far, take advantage of their action as acetylcholinesterase inhibitors [29]**.** Some of the drugs approved for therapeutic use show hepatotoxicity [31]**,** consequently there have been a continuous search for new safer natural agent in this respect. So, inhibitors of acetylcholinestarase activity are considered useful for the treatment of Alzheimer dementia [32, 33]. This is due to improvement of the level of acetylcholine transmitter that has been reported to be reduced in AD [34, 2].

Inflammation may play an important role in the pathogenesis of dementia. Studies reported an association between plasma levels of inflammatory markers and the risk of dementia and AD [35]. A variety of inflammatory proteins have been identified in brains of AD patients, including inflammatory cytokines, acute phase proteins and complement components [14, 36]. It was suggested that anti-inflammatory agent may have potential benefits towards AD [37].

Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity and anti-inflammatory [38, 39]. The antioxidant activity of phenolics is related to a number of different mechanisms such as free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and as acting as substrate for radical such as superoxide and hydroxide [40].

It has been reported previously that vitamin C, malic acid, citric acid and glucose are some of the possible antioxidant components in Carica [41]. Carica fruit has been shown previously to have antioxidative stress potential that was comparable to α-tocopherol [42]. In the present study both extracts of Carica fruits, leaves and seeds showed antioxidant activity which was the highest in case of methanol extract of Carica leaves (85%) and the lowest in case of methanol extract of the seed (37%). The antioxidant activity of methanol extract of leaves, fruits and seeds was proportional to their contents of phenolic compounds as shown in the present study. Five pigments (beta-carotene, lycopene, beta-cryptoxanthin, beta-cryptoxanthin myristoyl and lauroyl esters have been identified in the methanol extract of papaya [43], also Carica papaya was shown to contain α-tocopherol (111.3 mg/kg), these constituents may render papaya its antioxidant activity. The most potent anti-inflammatory activity in the present study was attributed to the methanol extract of Carica fruit among all the studied plants. Petroleum ether extract of Carica seed and leaves showed higher anticholineterase activity (73% and 65% respectively) than the other extracts of Carica papaya.

Origanum majorana L. herbs and their ethanol, n-hexane and supercritical CO_2 extracts have been shown previously to posses relatively strong antioxidant activity which has been attributed to phenolic contents [44]. In the present study antioxidant activity of methanol extract of Origanum was 74% while that of petroleum ether was 59%. While the acetylcholinesterase inhibition of the methanol extract in the present study was 44% and that of the petroleum ether extractwas only 23%. In a previous study it has been reported that ethanol extract of Origanum showed very high inhibitory effect on acetylcholinesterase which was ascribed to the presence of ursolic acid [32]. Ursolic acid, carnosic acid, and carnosol content of Origanum has also been reported to reduce oxidative stress [33, 44]. The phenolic content of Origanum in the current study showed high level (77.93 mg gallic acid equivalent/g dry sample) which support its effect as antioxidant. Origanum is rich in rosmarinic acid and hydroxycinnamic acid compounds which possess strong antioxidant activity [45]. In the present study both methanol and petroleum ether extract of Origanum showed low anti-inflammatory activity.

Thomson *et al.* [46] demonstrated in-vivo anti-inflammatory activity of ginger through reduction of prostaglandin E_2, an inflammatory mediator. It has been also reported that ginger extract may be useful in delaying the onset and the progression of neurodegenerative diseases specially AD through cell line assay. This was reflected through its anti-inflammatory and antioxidant effect [47]. Ginger has been shown to have potent antioxidant activity [48], which was claimed to be attributed to 6-gingerol a phenolic

compound that possess both antioxidant and anti-inflammatory activity [49]. It has also been demonstrated that biologically active constituents of ginger included vanillin, dihydroferulic acid, zingerone and ferulic acid [50]. Ginger was shown to have high concentration of melatonin; it also contains 6-dehydrogingerdione and curcumin which possess potent antioxidant effect [51, 52]. In the present study it has been shown that ginger not only possess anti-inflammatory and antioxidant activity but also acetylcholinesterase inhibiting activity which make it a good functional food for AD patients. Some of these activities may be related to its phenolic content that determined to be 29.29 mg gallic acid equivalent/g dry sample. Lipid fraction (petroleum ether extract) of ginger showed the highest anti-cholinesterase activity among the studied plant extracts. The unsaponifiable matter of lipid fraction of ginger in the current study was shown to contain campesterol, stigmasterol and sitosterol but in low percentage. Hydrocarbons of C15 represent 51.938% of the unsaponifiable matter.

Greg *et al*. [53] reported antioxidant and anti-inflammatory activity of grapes which have been attributed to the presence of polyphenol, flavonoids, beta-carotene, tocopherols and dietary fibers [54]. In the present study total phenolic contents of grape leaves was of the highest content (93.52 mg gallic acid equivalent/g dry sample) among the studied plants, however the content of the fruit was only 23.75 mg gallic acid equivalent/g dry sample. Grape leaves methanol extract ranked as having the second highest anti-inflammatory activity among the studied plants. Methanol extract of grape fruit also possess the third highest anticholinesterase activity (66%) among the studied plants. Extracts of grape leaves and fruits also showed high antioxidant activity ranged from 64-66%. In a previous study, Yilmaz and Toledo [55] reported the presence of important phytochemicals in grape skin (resveratrol, catechin, epicatechin, gallocatechin, gallic acid and ellagic acid) which possess potent antioxidant effect.

Fig fruits have been shown previously to have antioxidant activity, which correlated with total polyphenols, flavonoids and anthocyanins contents; cyanide-3-O-rhammoglucoside is the main anthocyanin in fig fruits [56, 57]. Total phenolic content of fig showed the lowest level (14.27 mg gallic acid equivalent/g dry weight) among the studied plants. Both extracts of fig showed anticholinesterase activity ranged from 45-64%. The anti-inflammatory activity of methanol extract was higher than that of the petroleum ether extract.

Conclusion. All the studied plants showed antioxidant, anti-inflammatory and anti-cholinesterase activity with variable degrees. The highest antioxidant and cholinesterase inhibiting activity were attributed to methanol and petroleum ether extract of ginger respectively. While methanol extract of Carica fruit was superior as anti-inflammatory agent. Combination of the previously mentioned extracts may have potential beneficial effect as functional food components towards Alzheimer' disease. The anti-inflammatory and antioxidant activity may be attributed to the presence of phenolic compounds in the studied plants.

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