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Antioxidative and Antimicrobial Properties Of Essential Oil Of Certain Aromatic Plants.

AL Zhrani M Othman[1], El-Halmouch Yasser[1,2], Mahmoud F Samy[1,3]*, and Gaafar M Ahmed[4].

[1]Biotechnology Department, Faculty of Science, Taif University, Taif 21974, Kingdom of Saudi Arabia.
[2]Botany Department, Faculty of Science, Damanhour University, Damanhour 22511, Egypt.
[3]Departement of Dairy Sciences and Technology, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
[4]Departement of special food and nutrition, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

The present study was performed to use essential oils of some medicinal herbs thyme (*Thymus vulgaris*), rosemary (*Rosmari nusofficinalis*), Fennel (*Foeniculum vulgare*), and Anise (*Pimpinella anisum*) as natural antioxidants and antimicrobial agents to increase shelf-life of mayonnaise. Essential oils and phenolic compounds of these aromatic plants were extracted and quantitative determined. Also the antioxidant and antimicrobial activity were determined. Essential oil of anise plant represents about 2.48%, while thyme had the highest content of total phenols (917.17 mg GAE/L). Essential oils were screened against two gram-negative bacteria (*Escherichia coli and Salmonella typhimurium*) and two gram positive bacteria (*Bacillus cereus and Staphylococcus aureus*) at four different concentrations using disc diffusion method. Both gram-positive and gram-negative bacteria were sensitive to the potent essential oils. In general, rosemary and thyme oils showed significant inhibitory effect against two bacterial species. Moderate effects were seen in other oil (anise and fennel). Also, the peroxide value of mayonnaise prepared with rosemary essential oil at 5ml/kg was significantly lower than the peroxide value of all systems during storage. Mayonnaise was treated by essential oils of selected herbs, then peroxide value during storage and sensory evaluation were determined. The results indicated that addition of essential oils of herbs prolonged the oxidative stability of mayonnaise and they can be used as antibacterial agents.

Keywords: Aromatic plant, Essential oil, Chemical composition, Antioxidant, Antimicrobial activity.

**Corresponding author*

INTRODUCTION

Preservation of food materials from degradation, mainly by oxidation processes or by microorganism activity, during production, storage and marketing is an important issue in the food industry. To achieve this purpose, the food industry has used synthetic additives (added or already present naturally in the foods), which diminish microbial growth or inhibit it. These additives may delay, in a significant way, the oxidation of easily oxidizable materials, such as fats. However, owing to the economical impact of spoiled foods and consumers' growing concerns over the safety of foods containing synthetic chemicals, much attention has been paid to naturally derived compounds or natural compounds [1]. Aromatic plants have been used for centuries as spices and condiments to confer aroma and flavor to food and beverages. Additionally, due to their constituents, medicinal and aromatic plants can act as stabilizer agents, playing an important role in the shelf-life of foods and beverages [2]. In the last decade scientific research has focused its interest on their essential oils and extracts as natural sources of antimicrobial and antioxidant compounds [3-7]. Essential oils are volatile, natural, complex compounds characterized by a strong odor. These are formed by aromatic plants as secondary metabolites [8]. The main advantage of essential oils is that they can be used in any food and are generally recognized as safe [9]. However, the stereochemistry, lipophilicity and other factors affected the biological activity of these compounds which might be altered positively or negatively by slight modifications [10]. With growing interest in the use of essential oils in the food, agricultural and pharmaceutical industries, examination of these natural products has become increasingly important. In recent years antibacterial and antifungal potential of essential oils have been extensively researched and reviewed [11-15]. Mayonnaise's manufacturers face a major problem of lipid oxidation which limits the shelf life of their products. Lipid oxidation occurring in food products is one of the major concerns in food technology. It is responsible for rancid odors and flavors of the products, with a consequent decrease in nutritional quality and safety caused by the formation of secondary, potentially toxic compounds. In order to control lipid oxidation of lipids and lipid-containing products, synthetic antioxidants have been used. This is very important to human health protection and also economically important. Nevertheless, toxicological effects of these synthetic antioxidants together with consumer preference for natural products have resulted in increased interest in the use and research of natural antioxidants from the point of view of safety [16-18]. Several studies associated with control of SE in food have been developed in laboratory scale and some already applied at the level of industrial production to ensure food security. Essential oils can be destroyed by heat treatment, but usually the salads with mayonnaise are not submitted to this type of treatment. This situation creates the need to use other methods for reduction or elimination of the pathogen within the concept of hurdle technology [19,12]

The aim of this work was to determine the yield content, total phenolic and antioxidant activity of essential oils of four spices widely cultivated in Egypt as: thyme, rosemary, Fennel and anise. Also, determine the effectiveness of the Egyptian essentials oils on the inhibition of the growth of some bacteria strains to determined that the spice essential oils could be used as natural food ingredients and represent a useful alternative for the food industry to reduce the quantity of synthetic additives used in their attempt to satisfy the demands of consumers.

MATERIAL AND METHODS

Plant material:

Thyme (*Thymus vulgaris*), rosemary (*Rosmari nusofficinalis*), Fennel (*Foeniculum vulgare*), and Anise (*Pimpinella anisum*) were obtained from local market in Aswan government, Egypt.

Solvent and standard reagents:

All solvents used throughout the whole work were of analytical grade. BHT was purchased from Sigma (St. Louis. MO. USA). 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and Folin-Cioculteau reagent was obtained from Gerbsaur Chemical Co. (German). Gallic acid (98%) was purchased from Aldrich Chemical Co. (ltd., England).

Extraction of essential oil

The essential oils of thyme, rosemary, fennel, and anise were extracted from entire plant (stems, leaves and flowers) by hydro-distillation using a Clevenger-type apparatus for 3 h. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate (0.5 g). The extracted essential oil was kept in sealed air-tight glass vials and covered with aluminum foil at 4ºC until further analysis.

Total phenol content

The total phenol content was determined using Folin- Ciocalteu's [20]. Values were expressed as gallic acid equivalent.

Antioxidant activity

Determination of antioxidant activity using 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging method, the antioxidant activity of four different concentrations (5, 10, 20 and 50 g/L) of essential oils were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical 2, 2-diphenyl-1-picryl-hydrazyl DPPH [21].

Microbiological Analysis:

Bacterial strain

Four bacterial strains of significant importance were used to test the antibacterial properties of the essential oils .Two of them were Gram-positive (*Bacillus cereus and Staphylococcus aureus*) and the others were Gram-negative (*E. coli* and *Salmonella typhimurium*). All strains were obtained from the Bacteriology lab. Ain shams hospital. The bacterial strains were examined microscopically and confirmed by biochemical tests. Bacterial strains were inoculated into Mueller Hinton broth (Difco) and incubated at 37^{o}C or 30 ^{o}C for 24h. The cultures were subjected to three successive 24-h. transfers before use. All cultures were adjusted to 106 CFU per ml prior to use.

Culture Media

Muller Hinton liquid and solid media (Difco) were used in this study. The liquid medium was sterilized by autoclaving at 121 oC for 20 min., and then used for sub- culture and optical density assay. Solid media was used for agar- well diffusion assay.

Well diffusion assay

Twenty ml of Muller Hinton agar was placed into 10ml Petri dishes and 0.1 ml of the active cultures was spread over the plate using a sterile glass spreader in order to get a uniform microbial growth for all plates [22]. A well was done using a 6mm diameter cork borer in the agar plate. The wells were filled with 10, 20 or 30 µl of the essential oil. All plates were sealed by Para film with sterile laboratory conditions to avoid evaporation of the agar plates. The plates were left for 30 min at room temperature to allow the diffusion of oil and then plates were incubated at 35-37ºC for 24 h. The inhibition zone was measured. Values were performed in triplicate and the mean value was recorded.

Technological methods:

Mayonnaise Preparation

Mayonnaise was prepared using the suggested formula according to Kishk [23] as following: salt (1.26 g), mustard (2.20 g) and white pepper (0.32 g) were mixed with fresh whole egg (22.17 g), vinegar (0.63 g) and lemon juice (2.20 g) using blender on low velocity for 5 sec; the previous mixture called the aqueous phase. The oil (70.0 g) was slowly added to the system during the first 30 sec. In order to evaluate the antioxidant effect of essential oils of thyme, rosemary, fennel and anise was added to the oil phase to make final concentrations in mayonnaise ranged from 0.5 and for comparison to the control samples and mayonnaise samples were stored at room temperature for two months.

Sensory Evaluation of mayonnaise:

Mayonnaise samples prepared using different concentrations of sage ethanolic extract were evaluated by ten staff members of Food Science Department, Faculty of Agriculture, Ain Shams University. They asked for evaluation of mayonnaise samples for color, odor, flavor, consistency, overall acceptability. Untreated mayonnaise sample was given as control. The mayonnaise samples were evaluated using the report sheet according to [24] as follows: No difference from reference sample = 5; more desirable than reference sample: slight = 6; moderate = 7; much =8; extreme = 9; less desirable than reference sample: slight = 4; moderate = 3; much = 2; extreme = 1. Additionally, the development of rancidity in mayonnaise samples was evaluated during storage period by the same staff members. They were also asked to smile the mayonnaise samples and score them according to the rancidity grading which was based upon four point intensity scale: 1 = no perception; 2 = weak; 3 = medium; 4 = extreme [25].

Peroxide value of mayonnaise samples:

The peroxide values were determined in triplicate samples by the extraction method of [26] Results expressed as milliequivalents/kg oil (meq. /kg oil).

Statistical analysis:

Statistical methods were used to calculate and standard deviations of three simultaneous assays carried out with the different methods. Analysis of variance (ANOVA) was applied to the data to determine differences ($P \geq 0.05$).

RESULTS AND DISCUSSION

Essential oil content

The essential oil content of the thyme, rosemary, Fennel and Anise are presented in Table (1). A highest content of essential oils were obtained in both anise and fennel (2.48 and 2.45%, respectively). They have no statistical difference at $p<0.05$. Our results are in agreement with Prabuseenivasan et al. [27] who demonstrated that anise herb have a high essential oil content. While, thyme and rosemary were seen to be a less rich source of essential oils (1.72 and 1.91%), respectively, [28] reported that The yields of the essential oils were fennel 2.5% and thyme 1.6%. Essential oils content could be used important indicator of the antioxidant and antimicrobial properties [12, 29].

The total phenolic compound content of the thyme (*T. vulgaris*), rosemary (*R. officinalis*), fennel (*F. vulgare*), and anise (*P. anisum*) essential oils are presented in Table (1). A high content of total phenols were obtained in anise and thyme. It was 905.8 and 917.2 mg GAE/L, respectively). Rosemary essential oil was seen to be a less rich source of total phenols (645.6 mg GAE/L), while fennel essential oil showed the lowest amount of total phenols (Table 1). Many authors [30-32], have described the potential antioxidant properties of polyphenols. These compounds act as antioxidants by donation of a hydrogen atom, as an acceptor of free radically, by interrupting chain oxidation reactions or by chelating metals. It has been suggested that the phenolic content of plant materials is correlated with their antioxidant and antibacterial activity. It is considered that the antioxidant activity of phenolic compounds is due to their high redox potentials, which allow them to act as reducing agents, hydrogen donors and single toxygen quenchers [33].

Table 1: Total phenols and essential oils content in thyme, rosemary, Fennel and Anise

Medicinal plants	Total phenols (mg/L)	Essential oil content (%)
Thyme	917.2^{ab}	1.72^{b}
Rosemary	645.6^{b}	1.91^{b}
Fennel	164.3^{c}	2.45^{a}
Anise	905.8^{a}	2.48^{a}

Different letters on the column for each parameter differ significantly at $p \leq 0.05$.

Table 2: DPPH scavenging activity (%) by different concentration of thyme, rosemary, fennel and anise essential oil.

Medicinal plants	DPPH scavenging activity (%) Oil concentration (µl/ml)				IC_{50}
	5	10	20	50	
Thyme	29.56 [a]	44.68 [a]	73.52 [a]	91.26 [a]	11.34[b]
Rosemary	16.42 [c]	27.46 [b]	51.25 [b]	74.93 [b]	19.74[b]
Fennel	3.69 [d]	6.28 [c]	10.49 [c]	16.14 [c]	159.59[a]
Anise	25.13 [b]	40.75 [ab]	69.98 [a]	88.47 [a]	14.65[b]

Different letters on the column for each parameter differ significantly at $p \leq 0.05$.

Antioxidant activity

There are many different methods for determining antioxidant function each of which depends on a particular generator of free radicals, acting by different mechanisms [34], For this reason, the antioxidant activity of thyme, rosemary, Fennel, and Anise essential oil was determined by DPPH spectrophotometric methods test. Table (2) shows the radical scavenging capacity of the spice essential oils tested using the "stable" free radical, DPPH. It can be seen that, the essential oils analyzed exhibited varying degrees of scavenging capacities. Anis and thyme essential oils showed the strongest radical scavenging effect (88.46 and 91.26%, respectively) at 50 mg/mL with no statistical difference. These activities were followed by the rosemary essential oil (74.93). While, fennel essential oil showed the lowest scavenging activity. The DPPH assay measures the ability of the extract to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity, which is reflected in a lower IC_{50} (The half maximal inhibitory concentration) [35] . The highest value of $IC_{50}$was obtained using fennel plant extraction (Table 2). [36-38], indicated that thyme essential oil was able to reduce the stable DPPH radical even better than BHT. This author demonstrated that the compound most responsible for this activity was the oxygenated monoterpenethymol as well as the mixture of mono- and sesquiterpene hydrocarbons. Thymol, with greater steric hindrance of the phenolic group in comparison to carvacrol had higher antioxidant activity [28].

Antimicrobial activity of the essential oils and phenolic compounds of medicinal herbs:

The antimicrobial activity of selected essential oils of thyme, rosemary, fennel and anise against four bacterial species are summarized in Tables 3. The results revealed that the selected essential oils of medicinal herbs showed antimicrobial activity with varying magnitudes. The zone of inhibition above 7mm in diameter was taken as positive result. Generally, all tested micro-organisms were sensitive to many of the essential oils of all herbs. The highest action was recorded by essential oil of thyme against *B. cereus*(67 mm) at 50µl/well. The highest inhibition zones were obtained at 50µl/well by thyme (Table 3). Concerning on rosemary essential oil, 10µl was enough to inhibit *S. aureus* growth. Inhibition zones formed by rosemary essential oil against *S. aureus* varied insignificantly from 10 to 50 µl. *S. typhimurium* was the most sensitive bacteria to fennel extract. Anise extract has the lowest effect against *B. cereus*, *S. aureus* and *E. coli.* Inhibition zones ranged from 6 to 38 mm in respect to the oil concentration. While, *S. typhimurium* was the most sensitive to Anise essential oil (Table 3). Both gram-positive and gram-negative bacteria were sensitive to the potent essential oils. In general, rosemary and thyme oils showed significant inhibitory effect against two bacterial species. Moderate effects were seen in other oil (anise and fennel). Plant essential oils have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies [39]. Essential oils are potential source of novel antimicrobial compounds [40] especially against bacterial pathogens. In vitro studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many herbs has been previously reviewed and classified as strong, medium or weak [41]. In our study rosemary and thyme essential oils exhibited strong activity against the selected bacterial strains. Several studies have shown that clove, sage and rosemary essential oils had strong and consistent inhibitory effects against various pathogens. In the present study, it was showed that fennel essential oil similar results were obtained by Takarada et al. [42].

Changes in peroxide value during storage of home mayonnaise:

Mayonnaise is susceptible to spoilage through the auto- oxidation of unsaturated and polyunsaturated fatty acids in oil. Lipid peroxidation, in food emulsions leads to the production of off-flavors and off-odors, thereby shortening the shelf life of these products [34]. The peroxide value (PV) value well established methods for determining oxidation products (both primary and secondary products) in fats and oils [44]. Changes in the PV of mayonnaise during storage at ambient temperature are illustrated in Table (4). Data revealed that PV values for all investigated samples were significantly increased as storage period increased compared with the control sample. The effect of natural essential oils of thyme, rosemary, fennel and anise on peroxide value of home mayonnaise samples over 2 month of storage periods under room temperature was illustrated in Table (4). Peroxide value range of 10-20 meq/kg. Food product is considered rancid but still acceptable. But if more than 20 meq/kg, it considered food product already rancid and unacceptable to consume [45-47].

All samples were able to maintain peroxide value less than 10 meq/kg until the sixty day of storage. Addition of antioxidants of essential oils from herbs (thyme, rosemary, fennel and anise) at various concentrations had significantly lower peroxide value than those of the control, throughout the duration of the study. However, the peroxide value of rosemary essential oil at 5ml/kg was significantly lower than the peroxide value of all systems during storage. On the other hand, addition of essential oils from fennel had significantly higher peroxide value of all samples. Meanwhile, the value of mayonnaise prepared with fennel essential oil had lower value compared to mayonnaise control.

Sensory evaluation of mayonnaise:

Mean scores for sensory evaluation of mayonnaise as affected by different concentrations of essential oil of thyme, rosemary, fennel and anise are shown in Table (5). Results indicated that the addition of these essential oils of herbs gave a better rating score in the mayonnaise samples under study than control samples. Generally, it could be observed that the addition of medicinal herbs essential oils to mayonnaise exhibited the highest characteristics of sensory evaluation and had higher addition of 0.5, 1.0, 2.0 and 5.0 ml/kg, respectively, to obtained good quality productive, suggesting their potential source for alternative dietary oils for manufactured mayonnaise. Increased security by increasing the concentration of essential oil in these products is not always possible, they may become sensory unacceptable. These data agree with [48]. Sensory evaluation showed that the addition of oregano essential oil at 0.6 or 0.9% in minced sheep meat was organoleptically accepted. Also [49] suggest that oregano essential oil provides additional protection able to increase the safety of vegetable salad with mayonnaise contaminated with SE, but not for those subjected to temperature abuse. From Food Safety point of view this study has identified the use of oregano essential oil like natural antimicrobial able to reduce the SE growth, an enteropathogen associated with food borne disease around the world in products derived from eggs such as mayonnaise.

CONCLUSION

The results obtained using different antioxidant methods showed that black cumin and thyme essential oil obtained from Egyptian plants can be considered good sources of natural compounds with significant antioxidant activity. However fennel essential oils are lowly effective to avoid the oxidation. Thyme essential oil presents the highest antibacterial activity of all essential oils studied followed by rosemary. The Gram-positive bacteria were most sensitive to the action of essential oil than the Gram-negative.

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Table 3: Antimicrobial activity of the extracted essential oils of Thyme, Rosemary, Fennel and Anise against *B. cereus, S. aureus, E. coli and S. typhimurium*

	Oil concentration (µl/well)															
	Thyme				Rosemary				Fennel				Anise			
	5	10	20	50	5	10	20	50	5	10	20	50	5	10	20	50
B. cereus	26^{c}	65^{a}	67^{a}	62^{a}	22^{b}	52^{b}	49^{b}	53^{b}	7^{b}	15^{b}	35^{b}	50^{c}	8^{b}	10^{c}	15^{c}	33^{b}
S. aureus	32^{b}	62^{a}	60^{a}	60^{a}	31^{a}	61^{a}	60^{a}	60^{a}	6^{b}	8^{c}	19^{c}	25^{d}	11^{b}	18^{b}	30^{b}	38^{b}
E. coli	19^{c}	32^{b}	59^{a}	64^{a}	7^{c}	16^{c}	33^{c}	30^{c}	28^{a}	50^{a}	59^{a}	61^{a}	6^{b}	8^{c}	25^{b}	30^{b}
S. typhimurium	58^{a}	65^{a}	62^{a}	64^{a}	9^{c}	19^{c}	38^{c}	33^{c}	35^{a}	50^{a}	63^{a}	59^{b}	29^{a}	44^{a}	57^{a}	61^{a}

Different letters on the column for each parameter differ significantly at $p \le 0.05$.

Table 4: Effect of different concentration of Thyme, Rosemary, Fennel and Anise on the peroxidase value (activity) of mayonnaise samples after one month of storage

Essential oil conc. (ml/kg)	peroxidasevalue (activity) of mayonnaise after different time of exposure to essential oils											
	Thyme			Rosemary			Fennel			Anise		
	0.0	One month	Two months	0.0	One month	Two months	0.0	One month	Two months	0.0	One month	Two months
0.5	1.06^{a}	5.36^{a}	10.27^{a}	1.04^{a}	5.02^{a}	9.48^{a}	1.05^{a}	8.83^{a}	15.65^{a}	1.08^{a}	6.54^{a}	13.14^{a}
1.0	1.04^{a}	4.86^{b}	8.16^{ab}	1.05^{a}	4.84^{a}	7.13^{bc}	1.06^{a}	7.43^{bc}	13.54^{a}	1.06^{a}	5.83^{ab}	11.52^{bc}
2.0	1.05^{a}	3.81^{c}	7.52^{bc}	1.05^{a}	4.23^{bc}	6.52^{cd}	1.05^{a}	5.28^{cd}	11.15^{a}	1.07^{a}	5.03^{bc}	9.18^{c}
5.0	1.09^{a}	3.56^{c}	5.69^{c}	1.08^{a}	3.07^{c}	4.21^{d}	1.04^{a}	4.46^{d}	10.36^{a}	1.05^{a}	4.74^{c}	6.52^{d}

Different letters on the column for each parameter differ significantly at $p \le 0.05$.

Table 5: Sensory evaluation of mayonnaise treated with different concentrations of Thyme, Rosemary, Fennel and Anise oils after two months of storage.

Essential oil conc. (ml/kg)	Sensory evaluation of mayonnaise																			
	Thyme					Rosemary					Fennel					Anise				
	Taste	Color	appearance	Odor	Overall acceptability	Taste	Color	Appearance	odor	Overall acceptability	Taste	Color	appearance	Odor	Overall acceptability	Taste	Color	appearance	odor	Overall acceptability
0.5	9.1^{a}	9.3^{a}	9.1^{a}	9.9^{a}	9.0^{a}	9.1^{a}	9.0^{a}	8.9^{a}	8.9^{a}	9.0^{a}	8.3^{a}	8.6^{a}	8.9^{a}	8.7^{a}	8.7^{a}	7.3^{a}	7.9^{a}	7.7^{a}	6.9^{a}	7.5^{a}
1.0	9.2^{a}	9.3^{a}	9.1^{a}	9.0^{a}	9.3^{a}	9.2^{a}	9.1^{a}	9.0^{a}	9.0^{a}	9.1^{a}	8.5^{a}	8.2^{ab}	8.7^{a}	9.0^{a}	8.7^{a}	6.3^{a}	7.4^{a}	6.8^{b}	5.4^{b}	6.6^{a}
2.0	8.9^{ab}	8.3^{b}	9.1^{a}	9.2^{a}	9.1^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.2^{a}	9.3^{a}	7.1^{b}	7.9^{b}	7.3^{b}	7.7^{bc}	7.4^{b}	5.4^{b}	6.6^{b}	5.6^{c}	4.8^{c}	6.2^{ab}
5.0	7.4^{b}	7.9^{c}	9.1^{a}	7.4^{b}	8.3^{b}	8.2^{b}	8.8^{b}	8.8^{a}	7.3^{b}	7.9^{b}	6.5^{b}	6.4^{c}	6.8^{c}	7.1^{c}	6.6^{c}	4.7^{c}	6.1^{c}	5.2^{c}	4.3^{c}	5.8^{b}

Different letters on the column for each parameter differ significantly at $p \le 0.05$.

The potential application of essential oils is of great interest in food, cosmetic and pharmaceutical industries since their possible use as natural additives emerged from the tendency to replace synthetic preservatives with natural ones. As conclusion, it is important to observe that the use of bio-preservatives (natural antimicrobials) is characterized as an additional barrier to the Good Manufacturing Practices (GMP) and HACCP program (Hazard Analysis Critical Control Point), fundamental to Food Safety.

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