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ANTIMICROBIAL PROPERTIES OF SOME PLANT EXTRACTS AGAINST VARIOUS FOOD-BORNE PATHOGENIC BACTERIA

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ABSTRACT

Food manufacturers and consumers demand additive-free, fresh and full-tasting food products while maintaining high standards of microbiological safety. The use of natural antimicrobial system for the preservation of foods could satisfy this demand. The use of certain plant extracts can guarantee a good microbiological safety in foods. There is a little quantitative data on antimicrobial activity of most plants extracts. Therefore, the growth of eleven food borne pathogenic bacterial strains; four Gram negative (*Enterobacter (Ent.) aerogenes, Escherichia (E.) coli, Pseudomonas (Ps.) aeruginosa* and *Ps. fluorescens*) and seven Gram-positive (*Bacillus (B.) cereus, B. firmus, B. pumilus, B. subtilis, Micrococcus (M.) luteus, M. varians* and *Staphylococcus (S.) aureus*) was studied in liquid media in the presence of some plant extracts (water and ethanolic) rich in total phenolic compounds, namely black tea, grape seed, green tea, rosemary and reference compounds (caffeine and catechin).

The ethanolic extracts of black tea, grape seed, green tea, and rosemary appear to be promising antibacterial agents and could be used in food industry to guarantee a good microbiological safety of foods.

Keywords: plant extracts, black tea, grape seeds, green tea, rosemary, growth inhibition, food pathogenic bacteria.

INTRODUCTION

Many plant extracts possess antimicrobial activities against a wide range of microorganisms related to food spoilage and safety (Friedman *et al.*, 2002 and Patrzykat & Douglas, 2003) besides their antioxidant properties (Basaga *et al.*, 1997) due to catechins (10–30%) and caffeine (1-5%), being major components of green or black teas (Shatta & Habiba, 1999; Shatta, .999; Beecher, 2003; Pan *et al.*, 2003 and Auger *et al.*, 2004) and considered to be responsible for the anticarcinogenic and antimutagenic properties of tea (Scott *et al.*, 1993; Kuroda & Hara, 1999, Zhu *et al.*, 2000; Cai *et al.*, 2002 and Gupta *et al.*, 2002). Among tea catechins, epigallocatechin gallate has been shown to have the strongest antimicrobial activity (Mabe *et al.*, 1999 and Amarowicz *et al.*, 2000).

The aqueous extract prepared from leaves of rosemary (*Rosmarinus officinalis* L.) is widely used as a folk remedy for abdominal colic (Al-Hader *et al.*, 1994) and marketed as powerful antioxidant of lipids in foods (Richheimer *et al.*, 1996). The major phenolic compounds are rosmarinic acid, glycosides of luteolin, carnosic acid, methylcarnosic and carnosol. The efficiency of the extract is improved in refrigerated foods, it withstands moderate heat treatment, therefore, it could be a useful preservative especially in refrigerated foods (Carlin *et al.*, 2000 and Del Campo *et al.*, 2000).

Also, grape seed extract contains simple phenolic acids (ρ-coumaric, cinnamic, caffeic, gentisic, ferulic and vanillic acids), trihydroxy stilbenes (resveratrol and polydatin), and flavonoids (catechin, epicatechin, and quercetin) in addition to oligomeric proanthocyanidin complex (OPCs). Hence, the seed offer an inexpensive source of OPCs (Fitzpatrick *et al.*, 1998).

The dramatic increase in the number of reported cases of food-borne illness necessitates the need for developing new and improved methods of food preservation. Due to negative consumer perceptions of artificial preservatives, attention is shifted towards alternatives that the consumers perceive as natural.

However, it remains to say that data on the sensitivity of pathogenic and spoilage bacteria to plant extracts are still limited, therefore, the aim of the present study is to evaluate the behavior of some plant extracts (black tea, grape seed, green tea and rosemary) on the survival and growth of some food borne pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Black and green tea leaves were obtained from a local market, rosemary leaves from the Horticulture Experimental Station, Faculty of Agriculture, Suez Canal University, Ismailia. Grape seeds were manually separated from *Vitis vinifera* variety Roumy Ahmer fruits at the laboratory.

Extraction

Preparation of the water extracts

Boiling water was added (300 ml) to tea leaves (50 g), and rosemary (35 g) separately in a 500 ml conical flask and stirred by a magnetic bar on a hot plate at 90 °C for 10 min. The extracts were filtered and analyzed in triplicate for their phenolic contents and their antibacterial capacity.

Preparation of ethanolic extracts

Ground air-dried rosemary, ground grape seed, green and black teas were macerated in ethanol (35 g / 300 ml 95% ethanol). The extracts were filtered through Whatman No. 1 filter paper in a Buchner funnel to remove coarse particles. The residue was re-extracted with ethanol 95%. The extracts were pooled and evaporated under vacuum at 40 °C.

Determination of total, free and conjugated phenolic contents

Total polyphenols (TP) and free polyphenols (FP) determined in the water and ethanolic plant extracts spectrophotomertically according to the Folin–Ciocalteau colorimetric method (Snell and Snell, 1953 and Singh *et al.*, 2002). Conjugated polyphenols (CP) were determined by difference (TP – FP). The amount of phenolic compouds was calculated from a standard curve of gallic acid (Sigma Chemical Co., St. Louis, Mo., USA) prepared at the same time. The results were expressed in gram(s) of gallic acid equivalents (GAE) per 100 g of extract (g GAE/100 g DM).

Antimicrobial preparations

Water and ethanolic extracts of black, green tea, rosemary and grape seed (ethanolic only) and two reference compounds catechin hydrate, 98%, (Sigma Chemical Co., St. Louis, Mo., USA), and caffeine anhydrous (Alfa Asar, A Johnson Matthey Company, 30 Bond Street, Word Hill MA 01835 8044, USA) were used for the experiments at the concentrations given in the Table (1). The solutions of additives were sterilized by filtration through a sterile 0.20 µm cellulose nitrate filter (Sartorius, AG. 37070 Goettingen, Germany) and then added at the selected concentrations (Table 1) to the growth media.

Table (1): The concentrations of plant extracts and reference compounds

Extract	Concentration (ppm) in medium
Black tea	
Water extract	500, 750 and 1000
Ethanolic extract	500, 750 and 1000
Grape seed	
Ethanolic extract	250, 500 and 1000
Green tea	
Water extract	500, 750 and 1000
Ethanolic extract	500, 750 and 1000
Rosemary	
Water extract	250, 500 and 1000
Ethanolic extract	250, 500 and 1000
Reference compounds	
Caffeine	125, 250 and 500
Catechin	250, 500 and 750

Microorganisms and culture media

The following strains and their source were as follows: Gram-negative bacteria: *Entrobacter (Ent.) aerogenes* ATCC 15050, *Escherichia (E.) coli* ATCC 15130, *Pseudomonas (Ps.) aeruginosa* DSM 50071 and *Ps. fluorescens* DSM 50090. Gram-positive bacteria: *Bacillus (B.) cereus* DSM 31, *B. firmus* ATCC 14575, *B. pumilus* ATCC 14884, *B. subtilis* DSM 10, *Micrococcus (M.) luteus* ATCC 15307, *M. varians* ATCC 15306 and *Staphylococcus (S.) aureus* ATCC 6538.

These strains were obtained from the American Type Culture Collection, Rockville, Maryland, USA (ATCC) and the German Collection of Micro-organisms, Braunschweig, Germany (DSM). All these strains were checked up and stored on Brain Heart Infusion (BHI, LAB M, Topley House, 52 Wash Lane, Bury, Lancashire, BL9 6AU, UK.) slants at 4 °C then sub-cultured twice in Brain Heart Infusion Broth (pH 7.4± 0.2) and incubated at 22 °C (for *Ps. aeruginosa* and *Ps. fluorescens*) and 37 °C (for the rest of strains) for 24 h before use.

Determination of bacterial growth and inhibition activity

Flasks of BHI broth containing various concentrations of additives (Table 1) and control (without additives) were inoculated with a priori prepared cultures at 1% level (initial counts, 10^6-10^7 cfu ml^{-1}) and incubated at 22 and 37°C. Triplicate flasks were treated for each additive at each concentration. The growth of each culture was monitored in two ways, by measuring its absorbance at 600 nm (OD_{600}) by a Spectronic 20D (Milton Roy Company, USA) at intervals for a total period of 72 hrs, and by plating on BHI agar (1.2% w/v) at 0, 3, 6, 9, 12, 24, 48, 72 hrs suitably diluted aliquots of the culture (viable counts). All experiments were repeated thrice.

Growth analysis

The growth percentage of 12 h culture equals

$$(OD_t\text{-}OD_{to})_{test} / (OD_t\text{-}OD_{to})_{control} \times 100,$$

Where: OD is the optical density at 600 nm, *t* is time after 12h, *to* is the initial time 0 h, *test* makes reference to the culture grown with additive(s) and *control* makes reference to the culture grown without additives (Nazer *et al.*, 2005). These variable indicates how much the growth is reduced in the presence of additives. A time of 12 h was chosen for the best discrimination of growth curves.

The inhibition percentage of the examined plant extracts was calculated as follows:

$$\text{Inhibition \%} = [\text{Log } N_2\text{-Log } N_1/\text{Log}N_2] \times 100,$$

Where: Log N_1: Log cfu ml^{-1} of the sample at the last hour (72^{nd} hr).

Log N_2: Log cfu ml^{-1} of control without additives at the last hour (72^{nd} hr)

Statistical analysis

The results are presented as means ± standard deviation from three replicates of each experiment. A P-value ≤ 0.01 is used to denote significant differences among mean values determined by analysis of variance (ANOVA) (CoStat program ver. 3.03, 1986).

RESULTS AND DISCUSSIOM

Results of the present study are given in Tables (2-5) and Figures (1-2).

Phenolic content

There is a wide range of phenolic (total, free and conjugated) concentrations in the plant extracts (water and ethanolic) analyzed as shown in Table (2). The values vary from 12.19 - 24.19, 7.73 - 18.96 and 4.46 – 5.49 g GAE 100 g^{-1} of water extracts from black tea, green tea and rosemary, respectively. While ethanolic extracts of the black tea, grape seed, green and rosemary were 19.20-36.66, 13.16-25.84 and 4.66-10.82 g GAE 100 g^{-1}, respectively Table (2). The difference between the extracts containing the lowest total phenolic content (rosemary water extract and grape seed ethanolic extract) and highest total phenolic content (black and green teas, ethanolic extract) was found to be more than 1.5-2-folds.

Table (2): Total, free and conjugated polyphenols in the tested plant extracts

Plant extracts	Polyphenols (g GAE) 100 g^{-1} extract		
	Total	Free	Conjugated
Black tea			
Water extract	24.19	18.96	5.23
Ethanolic extract	29.82	19.57	10.25
Grape seed			
Ethanolic extract	19.20	14.56	4.66
Green tea			
Water extract	21.28	15.79	5.49
Ethanolic extract	36.66	25.84	10.82
Rosemary			
water extract	12.19	7.73	4.46
ethanolic extract	22.73	13.16	9.57

Gram-negative bacteria

The growth percentage at 12 h was plotted versus concentration for each extract (Figs. 1 A and 2 A). The evolution of the growth percentage was not linear in most cases. The ethanolic extract of rosemary and grape seed led to a rapid decrease of the growth percentage, followed by green tea and black tea.

Tables (3 and 4) show that the water solutions of reference caffeine and catechin had no effect against *Ent. aerogenes* and *E. coli*. But the ethanolic extract of grape seed and rosemary give an inhibitory effect with the following inhibition percentages 55.59 and 75.95; 46.44 and 71.82, respectively at 1000 ppm.

Other strains, namely *Ps. aeruginosa* and *Ps. fluorescens* were more sensitive against ethanolic plant extracts as well as catechin. The inhibition percentages were 98.19 and 94.09 (black tea); 100 and 99.24 (grape seed), 88.49 and 80.40 (green tea) and 94.14 and 96.07 (rosemary). Caffeine did not show any effect, while catechin inhibited *Ps. fluorescens* by 77.99% at 750 ppm (Table, 4).

Gram-positive bacteria

Fig. 2B show that the growth percentage was not linear in most cases (ethanolic extracts) when the concentration of extracts increased. Water extracts as well as caffeine and catechin promoted the growth of some bacterial strains.

Water extracts and caffeine in most cases (*B. cereus*, *B. firmus*, *B. pumilus*, *B. subtilis* and *S. aureus*) showed no activity (Tables 4 and 5). Only, the water extracts of black and green tea exhibited an inhibitory effect against *M. luteus*, 85.39 and 67.23%, respectively at 1000 ppm.

Table (3): The effect of some plant extracts on the growth of some Gram-negative food borne pathogenic bacteria.

Items tested	Inhibition percent			
	Ent. aerogenes	*E. coli*	*Ps. aeruginosa*	*Ps. fluorescens*
Black tea, water extract 500 ppm	0.00± 0.01^{a}	0.00± 0.01^{a}	40.24 ± 0.51^{c}	28.05 ± 0.01^{a}
750 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	42.64 ± 0.52^{b}	28.55 ± 3.35^{a}
1000 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	46.64 ± 0.21^{a}	32.32 ± 0.01^{a}
ethanolic extract 500 ppm	10.86 ± 2.97^{b}	15.15 ± 0.01^{c}	70.89 ± 4.45^{c}	53.94 ± 4.85^{c}
750 ppm	16.45 ± 0.66^{b}	20.08 ± 0.38^{b}	89.04 ± 0.62^{b}	77.62 ± 0.01^{b}
1000 ppm	31.41 ± 3.79^{a}	31.25 ± 0.19^{a}	98.19 ± 1.82^{a}	94.09 ± 1.22^{a}
Grape seed ethanolic extract 250 ppm	23.69 ± 0.64^{b}	9.85 ± 0.01^{c}	72.94 ± 3.08^{b}	53.84 ± 0.79^{c}
500 ppm	24.67 ± 0.23^{b}	14.39 ± 0.01^{b}	85.00 ± 0.01^{b}	93.32 ± 0.58^{b}
1000 ppm	55.59 ± 0.73^{a}	46.44 ± 1.44^{a}	100.00 ± 0.01^{a}	99.24 ± 0.09^{a}
Green tea, water extract, 500 ppm	0.00± 0.01^{a}	0.00± 0.01^{a}	46.47 ± 2.98^{b}	33.84 ± 0.92^{c}
750 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	51.37 ± 1.18^{a,b}	39.94 ± 0.01^{b}
1000 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	56.37 ± 0.07^{a}	43.14 ± 0.46^{a}
ethanolic extract 500 ppm	0.00 ± 0.01^{b}	5.68 ± 3.41^{a}	63.77 ± 1.30^{c}	37.20 ± 0.61^{b}
750 ppm	18.42 ± 2.63^{a}	5.68 ±0.38^{a}	78.77 ± 1.39^{a,b}	69.76 ± 0.01^{a}
1000 ppm	19.74 ± 1.98^{a}	12.88 ± 2.27^{a}	88.49 ± 0.96^{a}	80.40 ± 1.07^{a}
Rosemary, water extract 250 ppm	2.43 ± 2.43^{a}	0.00± 0.01^{a}	0.00 ± 0.01^{c}	13.41 ± 2.44^{b}
500 ppm	5.21 ± 1.04^{a}	0.00 ± 0.01^{a}	5.56 ± 0.70^{b}	15.24 ± 1.83^{a,b}
1000 ppm	6.60 ± 3.82^{a}	0.00 ± 0.01^{a}	16.10 ± 1.72^{a}	20.12 ± 0.01^{a}
ethanolic extract 250 ppm	0.00 ±0.01^{c}	0.00± 0.01^{c}	43.49 ± 0.01^{c}	60.17 ± 4.06^{b}
500 ppm	27.30 ± 0.99^{b}	15.15 ± 0.76^{b}	59.32 ± 0.28^{b}	92.29 ± 2.35^{a}
1000 ppm	75.95 ± 5.83^{a}	71.82 ± 0.91^{a}	94.14 ± 3.67^{a}	96.07 ± 0.22^{a}

Within each column and for each extract, means having the same superscripts are not significantly different at p≤ 0.01

Table (4): The effect of caffeine and catechin on the growth of some food borne pathogenic bacteria.

Strains tested	Caffeine			Catechin		
	125 ppm	250 ppm	500 ppm	250 ppm	500 ppm	750 ppm
	Inhibition percent					
Gram-negative strain						
Ent. aerogenes	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{b}	3.57 ± 0.72^{a,b}	7.50 ± 2.50^{a}
E. coli	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	6.10 ± 5.09^{a}	6.50 ± 3.26^{a}	6.91 ± 3.66^{a}
Ps. aeruginosa	21.21 ± 0.02^{a}	23.29 ± 0.01^{a}	24.83 ± 3.60^{a}	35.45 ± 1.89^{b}	40.92 ± 3.60^{b}	49.45 ± 0.55^{a}
Ps. . fluorescens	0.00 ± 0.01^{b}	0.00 ± 0.01^{b}	5.90 ± 0.33^{a}	35.52 ±2.59^{c}	61.16 ± 0.31^{b}	77.99 ± 0.86^{a}
Gram-positive strain						
B. cereus	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	13.32 ± 2.10^{b}	14.25 ± 1.64^{b}	31.13 ± 0.24^{a}
B. firmus	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}
B. pumilus	0.00 ± 0.01^{b}	23.37 ± 5.05^{a}	25.36 ± 1.83^{a}	51.67 ± 0.22^{b}	54.79 ± 2.76^{a,b}	58.63 ± 1.67^{a}
B. subtilis	15.77 ± 0.39^{c}	20.50 ± 0.32^{b}	29.45 ± 0.83^{a}	27.69 ± 1.54^{b}	34.04 ± 1.35^{a}	37.92 ± 1.77^{a}
M. luteus	8.93 ± 1.79^{b}	13.10 ± 1.37^{b}	25.00 ± 1.79^{a}	45.54 ± 2.39^{a}	51.81 ± 1.91^{a}	58.39 ± 1.13^{a}
M. varians	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	34.58 ± 0.01^{c}	65.53 ± 0.01^{b}	76.25 ± 0.01^{a}
S. aureus	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	24.86 ± 1.63^{a}	26.13 ± 1.13^{a}	28.38 ± 0.28^{a}

Within each row and for each compound, means having the same superscripts are not significantly different at p≤ 0.01

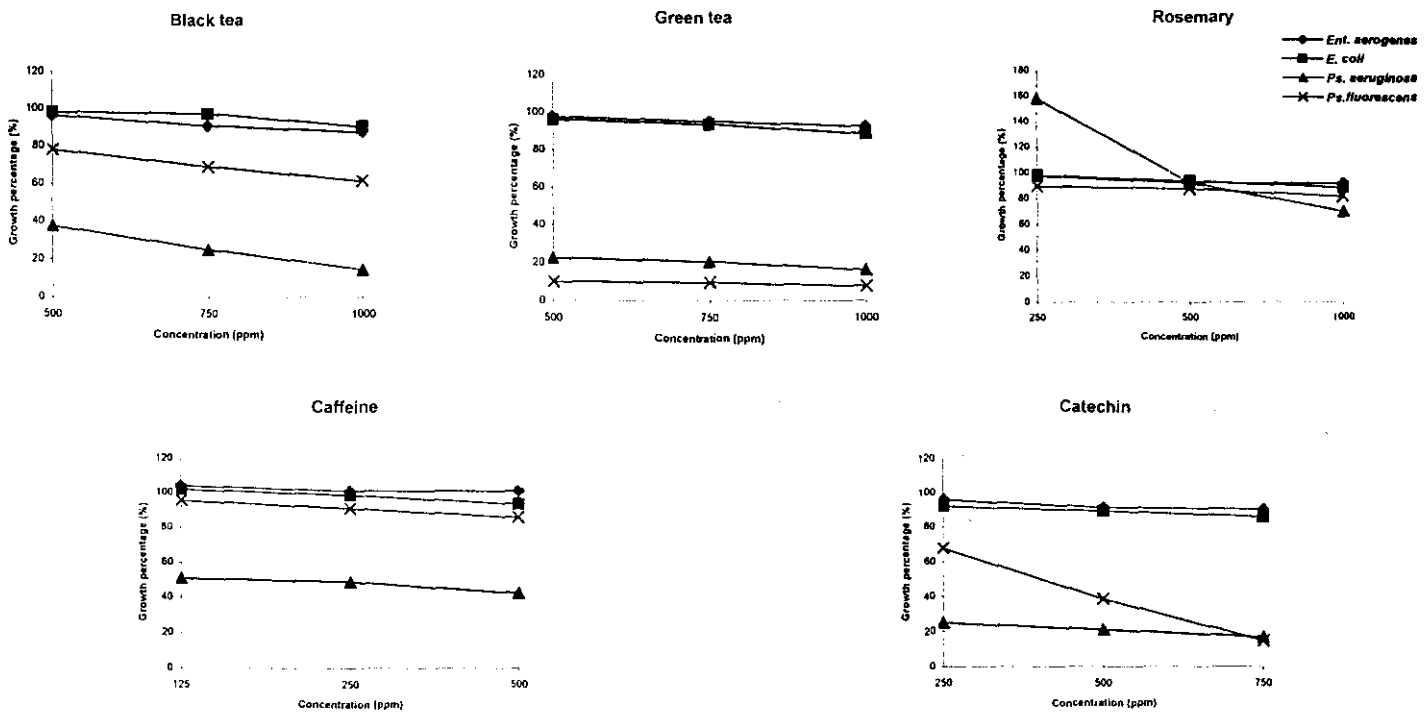

Fig (1 A): The effect of aqueous plant extracts (black, green and rosemary) and reference compounds (caffeine and catechin) on the growth percentage at 12 h of some Gram-negative food pathogenic bacteria.

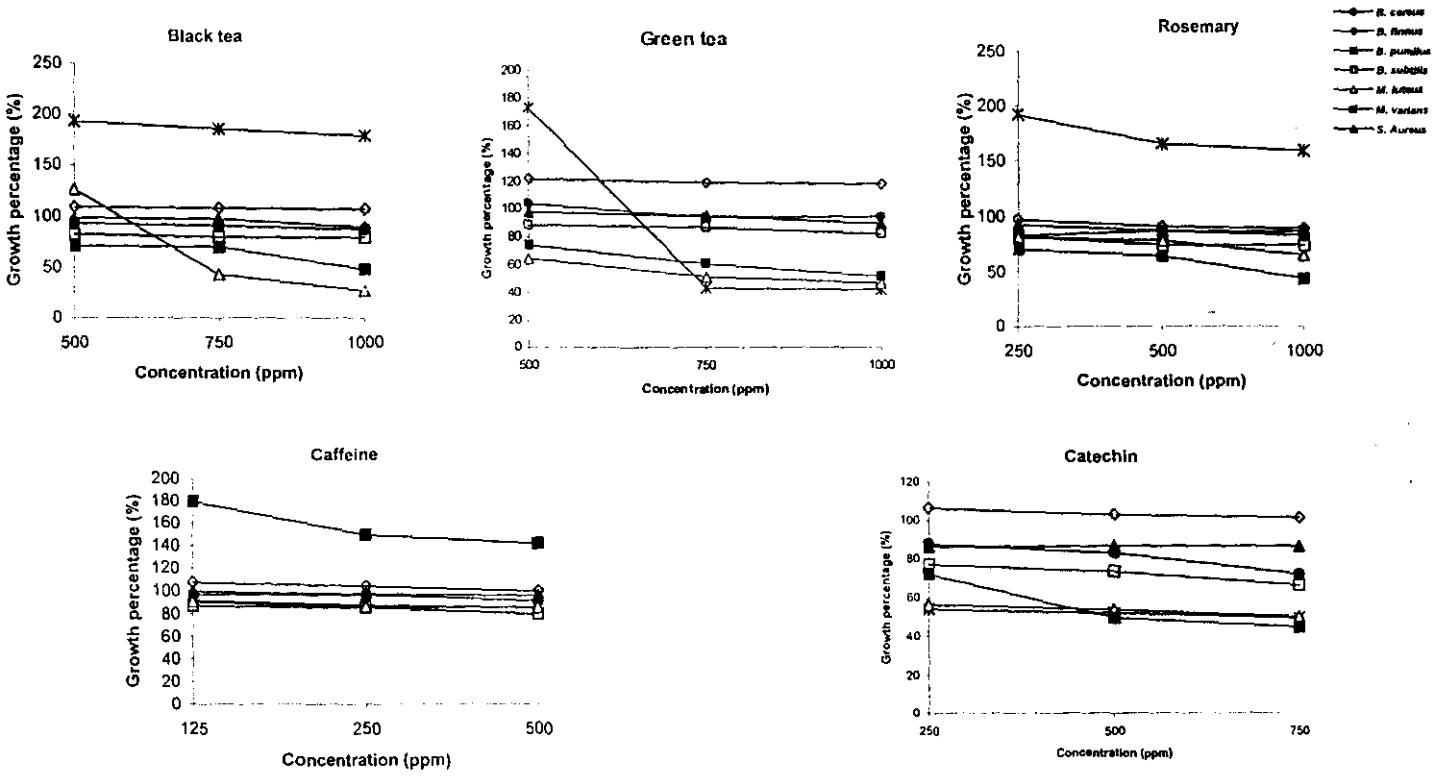

Fig (1 B): The effect of aqueous plant extracts (black, green and rosemary) and reference compounds (caffeine and catechin) on the growth percentage at 12 h of some Gram-positive food pathogenic bacteria.

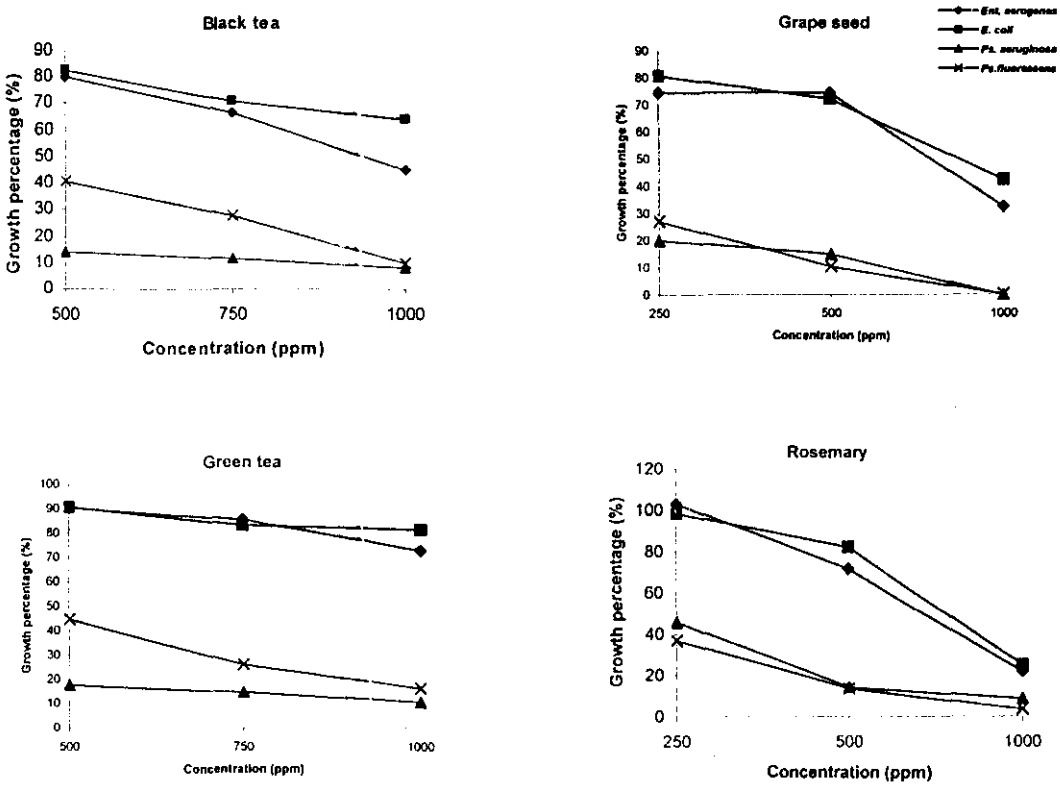

Fig (2 A): The effect of ethanolic plant extracts (black, grape seed, green and rosemary) and reference compounds (caffein and catechin) on the growth percentage at 12 h of some Gram-negative food pathogenic bacteria Growth

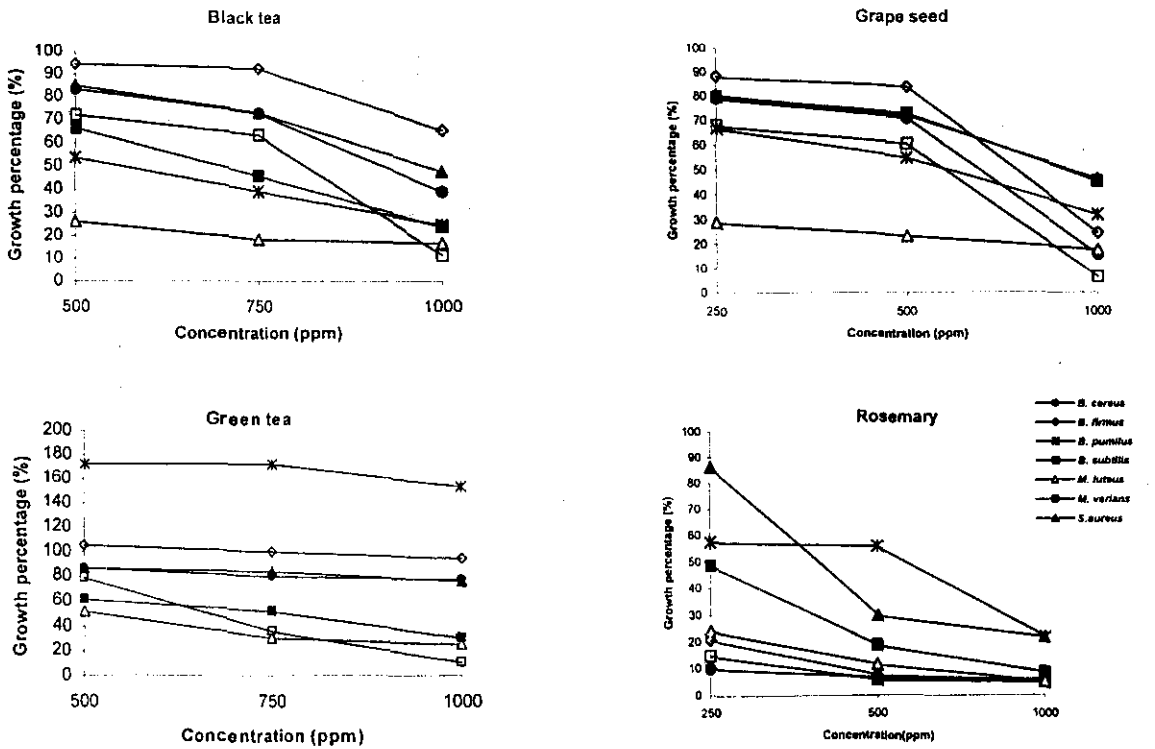

Fig (2 B): The effect of ethanolic plant extracts (black, grape seed, green and rosemary) and reference compounds (caffeine and catechin) on the growth percentage at 12 h of some Gram-positive food pathogenic bacteria Growth

Table (5): The effect of some plant extracts on the growth of some Gram-positive food borne pathogenic bacteria.

Items tested	Inhibition percent						
	B. cereus	*B. firmus*	*B. pumilus*	*B. subtilis*	*M. luteus*	*M. varians*	*S. aureus*
Black tea, water extract 500 ppm	0.00 ± 0.01^{b}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	7.31 ± 3.85^{b}	0.00 ± 0.01^{c}	35.83 ± 2.71^{b}	0.00 ± 0.01^{a}
750 ppm	0.00 ± 0.01^{b}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	10.38 ± 0.39^{a,b}	32.44 ± 0.30^{b}	42.12 ± 5.47^{a,b}	0.00 ± 0.01^{a}
1000 ppm	13.51 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	18.26 ± 2.87^{a}	85.39± 0.51^{a}	48.75 ± 1.46^{a}	0.00 ± 0.01^{a}
ethanolic extract 500 ppm	22.29 ± 1.58^{b}	8.41 ± 0.65^{b}	64.86 ± 2.54^{b}	33.27 ± 2.12^{c}	66.85 ± 0.78^{b}	48.75 ± 2.18^{b}	26.49 ± 0.54^{c}
750 ppm	29.73 ± 2.52^{b}	8.58 ± 1.91^{b}	66.30 ± 1.82^{b}	40.92 ± 1.70^{b}	67.92 ± 0.90^{b}	67.08 ± 1.09^{a,b}	38.11 ± 0.27^{b}
1000 ppm	63.79 ± 2.41^{a}	64.47 ± 1.31^{a}	83.77 ± 2.83^{a}	58.64 ± 3.35^{a}	85.21 ± 0.45^{a}	73.85 ± 3.13^{a}	56.89 ± 3.11^{a}
Grape seed ethanolic extract 250 ppm	31.08 ± 3.87^{c}	11.90 ± 0.01^{b}	56.45 ± 1.09^{c}	39.62 ± 2.70^{c}	71.19 ± 0.01^{c}	64.01 ± 2.55^{b}	33.96 ± 2.17^{b}
500 ppm	61.62 ± 0.01^{b}	14.76 ± 0.01^{b}	63.77 ± 1.02^{b}	45.00 ± 0.33^{b}	78.69 ± 0.48^{b}	67.14 ± 1.72^{b}	38.11 ± 2.42^{b}
1000 ppm	95.68 ± 2.17^{a}	74.25 ± 4.95^{a}	81.38 ± 0.45^{a}	93.15 ± 0.77^{a}	83.48 ± 2.41^{a}	80.52 ± 3.547^{a}	67.03 ± 1.62^{a}
Green tea, water extract, 500 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	9.23 ± 0.01^{a}	30.85 ± 0.62^{c}	30.73 ± 1.56^{c}	0.00 ± 0.01^{b}
750 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	10.77 ± 2.31^{a}	55.15 ± 0.70^{b}	37.92 ± 0.21^{b}	0.00 ± 0.01^{b}
1000 ppm	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	13.85 ± 5.39^{a}	67.23 ± 1.08^{a}	46.77 ± 2.40^{a}	6.49 ± 0.54^{a}
ethanolic extract 500 ppm	13.06 ± 1.35^{b}	0.00 ± 0.01^{a}	0.00 ± 0.01^{c}	26.54 ± 0.77^{c}	28.57 ± 1.19^{c}	46.88 ± 0.42^{b}	17.84 ± 2.16^{b}
750 ppm	15.99 ± 3.83^{b}	0.00 ± 0.01^{a}	69.63 ± 0.35^{b}	39.31 ± 0.23^{b}	31.55 ± 0.01^{b}	54.17 ± 0.01^{b}	20.41 ± 1.37^{b}
1000 ppm	23.87 ± 1.81^{a}	0.00 ± 0.01^{a}	75.22 ± 0.65^{a}	90.19 ± 0.81^{a}	44.05 ± 0.60^{a}	87.08 ± 0.73^{a}	32.70 ± 0.27^{a}
Rosemary, water extract 250 ppm	9.81 ± 1.87^{a}	0.00 ± 0.01^{b}	0.00 ± 0.01^{a}	15.00 ± 1.92^{a}	16.67 ± 5.96^{a}	47.71 ± 2.51^{a}	7.78 ± 0.01^{b}
500 ppm	13.08 ± 1.14^{a}	0.00 ± 0.01^{b}	0.00 ± 0.01^{a}	22.31 ± 0.77^{a}	22.62 ± 0.01^{a}	53.32 ± 3.23^{a}	10.97 ± 0.91^{b}
1000 ppm	13.08 ± 0.47^{a}	4.29 ± 1.91^{a}	0.00 ± 0.01^{a}	23.55 ± 4.23^{a}	25.00 ± 1.19^{a}	59.27 ± 0.31^{a}	17.96 ± 1.80^{a}
ethanolic extract 250 ppm	89.32 ± 4.55^{a}	83.22 ± 1.48^{c}	85.94 ± 0.85^{a}	78.81 ± 0.04^{c}	77.98 ± 1.31^{a}	78.96 ± 5.29^{b}	30.06 ± 4.35^{b}
500 ppm	91.81 ± 3.42^{a}	91.39 ± 1.96^{b}	88.84 ± 2.32^{a}	87.35 ± 2.20^{b}	84.94 ± 0.89^{a}	90.00 ± 4.77^{a,b}	80.89 ± 2.80^{a}
1000 ppm	93.87 ± 0.72^{a}	96.89 ± 0.49^{a}	89.46 ± 2.07^{a}	93.00 ± 0.15^{a}	90.93 ± 0.32^{a}	93.49 ± 0.01^{a}	90.05 ± 2.27^{a}

Within each column and for each extract, means having the same superscripts are not significantly different at p≤ 0.01

The ethanolic extracts of grape seed and rosemary in particular exhibited an inhibitory effect and a clear selectivity towards the Gram-positive microorganisms. Among the extracts, the ethanolic extract of rosemary was the most efficient followed by grape seed, black and green teas. The data given in Table (5) indicate a good antibacterial activity of ethanolic grape seed and rosemary extracts against *B. cereus* (95.68 and 93.87%), *B. firmus* (74.25 and 96.89%), *B. pumilus* (81.38 and 89.46%), *B. subtilis* (93.15 and

93.00%), *M. luteus* (83.48 and 90.93%), *M. varians* (80.52 and 93.49%) and *S. aureus* (67.03 and 90.05%) at 1000 ppm, respectively.

Again, some inhibitory effect of the ethanolic black and green teas extracts appeared. The inhibition percentages of black tea ethanolic extract were 63.79 (*B. cereus*), 64.47 (*B. firmus*), 83.77 (*B. pumilus*), 58.64 (*B. subtilis*), 85.21 (*M. luteus*) and 56.89 (*S. aureus*). For the green tea ethanolic extract, the inhibition percentages were 75.22 (*B. pumilus*), 90.19 (*B. subtilis*) and 87.08 (*M. varians*). These results agree with Hara and Ishigami (1989) who reported that Japanese green tea had antibacterial activity against *S. aureus* and *B. cereus*. Also, Del Campo *et al.* (2000) reported that Gram-positive bacteria were more sensitive to bactericidal effect of green tea catechins, than Gram-negative bacteria. Moreover, the same authors reported that lipopolysaccharides forming the cell wall of Gram-negative bacteria presumably acted as a barrier to the penetration of phenolic compounds. But, Ikigai *et al.* (1993) clamied catechins disrupt cell membrane integrity, causing leakage from liposomes.

Conclusively, the antibacterial activity of aqueous extracts is considerably lower than that of the ethanolic extracts. These results are in agreement with Pandit and Shelef, (1994) who reported that the antilisterial activity of the ethanolic extract of rosemary was higher than that of the aqueous extract. Moreover, Del Campo *et al.* (2000) reported that ethanolic extracts seemed to be the most active against most of the strains. Rosemary and grape seed extracts have a promising antibacterial effect that could be used in food industry. This may lead to a renewed interest in the use of natural products (grape seed and rosemary) as decontaminants.

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تأثير بعض المستخلصات النباتية على بعض البكتيريا الممرضة فى الغذاء

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درس تأثير بعض المستخلصات النباتية (مائية و كحولية) الغنية بما تحويه من فينولات مثل (الشاى الاسود والاخضر والحصالبان وبذور العنب) على نشاط أحدى عشرة سلالة بكتيرية ممرضة فى الغذاء اربع منها سالبة لجرام وهى (*Enterobacter (Ent.) aerogenes, Escherichia (E.) coli,* وسبع سلالات موجبة لجرام وهى *Pseudomonas (Ps.) aeruginosa* and *Ps. fluorescens*) (*Bacillus (B.) cereus, B. firmus, B. pumilus, B. subtilis, Micrococcus (M.) luteus, M. varians, Staphylococcus (S.) aureus*) وذلك فى مرق Brain Heart Infusion محتوي على تركيزات مختلفة من المستخلصات النباتية السالفة الذكر. وللمقارنة أختير كل من الكافيين والكاتشين كاحد المركبات الرئيسية الموجودة فى تلك المستخلصات لدراسة تأثيرهما على نمو ونشاط هذه الميكروبات.

كان للمستخلصات الكحولية النباتية المختبرة تأثير مثبط واعد على نمو الميكروبات المختبرة، بالاخص مستخلصا الحصالبان وبذور العنب والتى يمكن أستخدامهما فى صناعة الغذاء.

فى ضوء النتائج يمكن التوصية باستخدام بعض المستخلصات النباتية كمواد طبيعية لتوفير امان ميكروبى جيد للغذاء وهذا ما يتطلبه المستهلك دون تأثير سلبى على صحته و كذا دون تغير فى جودة الغذاء وذلك بالمقارنة باستخدام المواد الحافظة الصناعية.