Study the effect of coffee and cardamom on the viability of some probiotic strains and their cytotoxicity

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Abstract: The aim of this research was studying the effect of coffee and cardamom on the viability of some probiotic strains and their cytotoxicity, also determining the optimal percentage for use of each of them. 5% coffee inhibits the growth of all probiotic strains (*Bifidobacterium bifidum; Bifidobacterium breve; Bifidobacterium infantis; Lactobacillus acidophilus; Lactobacillus rhamnosus; Lactobacillus reuteri and Enterococcus faecium)*. No growth was observed at 7.5% coffee or more except *Ent. faecium.* 1% cardamom had moderate effect on the viability of probiotic strains. While 3% or more completely inhibit all probiotic strains. Also, cardamom extract at different concentrations had inhibitory activity on *E. coli, Salmonella typhi , S. aureus, and Bacillus cereuss* and the coffee extract had inhibitory activity on all strains at 5%, however no inhibitory activity was observed against *E. coli,* or *Salmonella typhi* at 2.5%. *S. aureus* has been identified as the most sensitive strain against cardamom and coffee. Cardamom and coffee have a strong influence as anticancer cells in the liver and acceptable effect in the colon.

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1. Introduction

The word 'probiotic' comes from Greek language 'pro bios' which means 'for life' opposed to 'antibiotics' which means 'against life'. More than 400 bacterial species exit in human intestinal tract. It is an enormously complex ecosystem that includes both facultatively anaerobic and anaerobic microorganisms (Naidu *et al.,* 1999). The numbers of genera is nearly steady, because they each have their own growth niches (Fooks, *et al.,* 1999). The composition of the gut microflora is constant but can be affected by some factors such as; age, diet, environment, stress and medication (Fuller, 2007). To have a healthy intestine the balance of the bacteria must be maintained but this is difficult as the lifestyles change.

Lots of factors may change the balance away from potentially eneficial or health promoting bacteria like lactobacilli and bifidobacteria to potentially harmful or pathogenic microorganisms like clostridia, sulphate reducers and *Bacteroides* species. It makes the host more susceptible to the illnesses. In this case the prevalence of the beneficial bacteria must be supported. Using of probiotics help to protect the host from various intestinal diseases and disorders while increasing the number of beneficial bacteria and make the balance steady again (Fooks, *et al.,* 1999).

Arabic coffee is a popular beverage in Saudi Arabia is prepared using quantities of coffee and cardamom in a ratio of 4 coffee: 1 cardamom. Coffee (*Coffea Arabica*) has scientific interests because it is a rich source of a number of phenol compounds with antioxidant effects *in vitro* and contains several species of xanthines such as caffeine, teobromine and theophylline (Hostettmann, 2000 and Nardini, 2002). Main polyphenols in coffee are chlorogenic acids such as caffeic, ferulic, and p-coumaric acid, caffeoylquinic acid, with 5-O-caffeoyl-quinic acid (Arts, 2000 and Clifford, 2004). Meta-analyses have concluded that moderate to high coffee consumption (three to six cups/day) is not significantly associated with an increased risk of coronary death or heart attack (Kuriyama, 2006 and Thielecke, 2009). In addition, habitual coffee drinking has been associated with the prevention of diseases including cancer, cardiovascular disorders, obesity and diabetes as well as neurodegenerative disorders (Kuriyama, 2006; Hu, 2007; Eskelinen, 2009; Thielecke, 2009; Ogunleye, 2010; Choi, 2011). Cardamom (*Elettaria cardamomum* Maton), the Queen of all spices has a history as old as human race (Padmakumari, 2010). Cardamom is the fruits which are commonly known as (Heel khurd). It is used in Unani and Indian system of medicine to treat gastrointestinal disorders ,carminative, stomachic, diuretic, abortificient, antibacterial, antiviral, antifungal and is considered useful in treatment of constipation, colic, diarrhea, dyspepsia,

vomiting, headache, epilepsy and cardiovascular diseases ,blood pressure lowering ,relieve gas, abdominal distention, belching nausea, heartburn, anticancer and antidiabetic properties (Khan and Rahman, 1992, Ilangantileke *et al.,* 1993, Duke *et al.,* 2002 ,Jamal *et al.,* 2006, Gilani *et al.,* 2007 and El-yamani, 2011).Cardamom has antioxidant properties and can increase levels of glutathione and antioxidant enzymes in the body (Verma, 2010 and Bisht, 2011).

The aim of this research was studding the effect of coffee and cardamom on the viability of some probiotic strains and their cytotoxicity, also determining the optimal percentage for use of each of them.

2. Material and methods:

Preparation of coffee and cardamom extract medium

Roasted coffee beans were ground, added to distilled water and heated at 100° C for 5 minutes. The extract was filtered through Whatman No.1 filter paper and used to dilute plate count agar. After sterilization at 121 ° C for 20 minutes, the agar was poured into sterile petri plates to make agar plates. Agar plates containing various concentrations of coffee (2.5, 5.0, 7.5 and 10.0 g per 100 mL plate count agar) or cardamom (1, 2, 3, 4 g per 100 mL plate count agar) were used to study the effect of coffee or cardamom on the growth of probiotic strains. The extracts were also used to study the antibacterial susceptibility on some pathogenic strains with the same concentration (2.5, 5.0, 7.5 and 10.0 g or 1, 2, 3, 4 g per 100 mL for coffee or cardamom respectively by adding distilled water).

Probiotic strains:

Bifidobacterium bifidum; Bifidobacterium breve; Bifidobacterium infantis; Lactobacillus acidophilus; Lactobacillus rhamnosus; Lactobacillus reuteri and Enterococcus faecium were obtained from Dairy Microbiology lab, National Research Center – Cairo, Egypt.

Pathogenic bacteria:

Escherichia coli O157:H7, Salmonella Typhi were obtained from Microbiology laboratory, National Research Center, Cairo, Egypt. *Staphylococcus aureus* ATCC 6538, *Bacillus cereuss* B-3711 was obtained from NRRL (North Regional Research Laboratory, Illinois USA).

Effect of coffee and cardamom on friendly bacteria (Probiotic bacteria):

In order to assess coffee and cardamom on friendly bacteria (probiotic), each strain was grown in Trepton soy broth at 37'C for 24 hours and after incubation enumerated on Trepton soy agar plates containing 0, 2.5, *5.0,* 7.5 and 10.0 g or 1, 2, 3, 4 g of coffee or cardamom extracts respectively per 100 mL medium after incubated at 37 C *for* 48 hours.

Antimicrobial activity test

All strains used in the study were inoculated to TSB agar and incubated at 37±0.1° C for 24 hrs and were allowed to grow until they reach 10^8-10^9 cfu/ml. The 0.1 ml of inoculum from the prepared culture was transferred to Mueller-Hinton Agar medium. The inoculum was spread to surface of plates with a sterile swab and the inoculated plates were dried at room temperature. Paper discs (5mm) embedded within each dilute from plant extracts were placed on previously inoculated plates and were incubated at 37±0.1° C for 48 hrs. After incubation the zones of growth inhibition around disks were measured in mm (Anonymous, 1997). Antibacterial activity studies were carried out for each test strains in duplicate and average measurement were calculated.

Cytotoxicity Effect on Different Cell Lines (HePG2 and HCT116):

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan (Freshney, 2000).

Procedure:

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 hrs under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with a sample at concentration of 100 µg/ml (primary screening). Potent plant extracts from the primary bioassay were subjected to secondary screening for the determination of their LC_{50} and LC_{90} using different descending concentrations (100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml). Cells were suspended in RPMI 1640 medium(for HePG2, HCT116) and DMEM media (for MCF7), 1% antibioticantimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine in 96-well flat bottom microplate at 37 ºC under 5% CO_2. After 48 hrs of incubation, medium was aspirated, 40µl MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added

to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/ml of *Annona cherimolia* extract was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions (Hughes, *et al*., 1997).

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%.

The percentage of change in viability was calculated according to the formula:

(Absorbance of extract / Absorbance of negative control) -1) x 100

A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

3. Results and Discussion

Results in table (1) show the effect of coffee and cardamom extracts on the viability of some probiotic strains. The results demonstrated that the 5% coffee inhibits the growth of all probiotic strains. No growth was observed at 7.5% coffee or more except *Ent. faecium.* This may be due to the coffee beans content, except caffeine, a very strong, not identified yet, antibacterial agent [Daglia *et al.,* 1998 and Duda-Chodak *et al.,* 2008], which is harmful towards gram-positive and gram-negative bacteria. So, addition high percentage from coffee to the food products that include gram-positive as *Lactobacillus or Bifidobacterium* can cause the fall in the bacteria viability.

In the same table, we noticed that 1% cardamom had moderate effect on the viability of probiotic strains, while the count of probiotic strains were dramatically decrease at 2%. 3% or more completely inhibit all probiotic strains. The results agree with Bayoum (1998) & *Sarabi-Jamab and Niazmand (2009).*

Table (1): Effect of coffee and cardamom on the count of some probiotic strains.

Strains	Control	Coffee				Cardamom			
		2.5%	5%	7.5%	10%	1%	2%	3%	4%
B. bifidum	$4x10^6$	$1x10^5$	$2.1x10^3$	NG	NG	$3.1x10^4$	2.4x10	NG	NG
B. breve	$7.1x10^6$	$41x10^5$	$6.3x10^2$	NG	NG	$2.1x10^4$	3.7x10	NG	NG
B. infantis	$16x10^6$	$2.3x10^4$	$7.2x10^3$	NG	NG	$2.3x10^3$	NG	NG	NG
Lact. acidophilus	$8.3x10^6$	$3.1x10^5$	$3.5x10^3$	NG	NG	$1.1x10^4$	5.2x10	NG	NG
Lact. rhamnosus	$37x10^6$	$6x10^5$	$8.0x10^3$	NG	NG	$7.6x10^4$	2x10	NG	NG
Lact. reuteri	$94x10^6$	$1.9x10^4$	$3.9x10^3$	NG	NG	$4.1x10^3$	3.3x10	NG	NG
Ent. faecium	$154x10^6$	$2x10^6$	$9.4x10^5$	$6.2x10^3$	*NG*	$5.2x10^5$	$7.1x10^2$	*NG*	*NG*

Table (2): Antibacterial activities of Coffee and Cardamom extracts against some pathogenic bacteria.

Strains	Coffee				Cardamom			
	2.5%	5%	7.5%	10%	1%	2%	3%	4%
Escherichia coli O157:H7	-	6	9	13	8	9	10	11
Salmonella Typhi	_	6.5	10	15	10	12	13	15
Staphylococcus aureus	7	13	19	27	15	17	19	22
Bacillus cereuss	6.5	10	17	23	9	10	12	14

Results of antimicrobial activity assays are represented in Table 2 show that the antimicrobial activity assays indicated that cardamom extract at different concentration had inhibitory activity on *E. coli, Salmonella typhi , S. aureus, and Bacillus cereuss*. Also we noticed that the coffee extract had inhibitory activity on all strains at 5%, however no inhibitory activity was observed against *E. coli,* or *Salmonella typhi* at 2.5%. *S. aureus* which is an important pathogen in food-poisoning has been identified as the most sensitive strain against cardamom and coffee.

In this study, extract of coffee and cardamom seed displayed a variable degree of antimicrobial activity on different microorganisms. *S. aureus* was found to be more sensitive strain than the others. Antimicrobial characteristics of the plant are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue (Baytop, 1984 and Con, *et al.,* 1998). These results agree with Fardiaz (1995) and Sema, *et al.* (2005)

Cytotoxic Activity of Selected Extracts on Different Cell Lines:

The MTT is well established method used to assess mitochondrial competence (Freshney, 2000). In case of HePG 2 cell line (liver), the cardamom aqueous extract showed the best result (96.9%) and

gave moderate activity on HCT116 cell line (colon) (66.8%). While coffee extract had no activity on HCT116 cell line (12.5%) and had good cytotoxic activity on liver cell line (76%) as shown in Table 3.

Table (3) Safety of using coffee and cardamom aqueous extract (100-0.78mg/ml) on human tissues using cytotoxic activity test measuring MTT

Tissues	Cytotoxicity (%) at 100 µg/ml (coffee)	Cytotoxicity (%) at 100 µg/ml (cardamom)
Colon	12.5%	66.8
Liver	76%	96.9%

In conclusion, our results indicated that extract of the cardamom seed and coffee had a strong inhibitory activity on some pathogens. According to us, using cardamom as antimicrobial additives in food may be useful.

The reduction in the viability of probiotic bacteria in the medium containing 7% coffee extract or 3% cardamom was due to antimicrobial compounds.

From these results, we recommend not using a higher proportion of 5% coffee and 2% Cardamom as these ratios have an impact on especially pathogenic bacteria that cause food poisoning and have no effect on beneficial bacteria.

And also have a strong influence as anticancer cells in the liver and acceptable effect in the colon. To be consideration that this ratio is already being used in Arabic coffee making.

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References

1. Anonymous (1997). NCCLS Performance Standards for Antimicrobial Disk Susceptibility Tests. Sixth Ed., Approved Standard, M2-A6, 17(1), Replaces M2-A5, 13(24).
2. Arts IC; van De Putte B; Hollman PC (2000). Catching contents of foods commonly consumed in the Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. J Agric Food Chem., 48: 1752–1757.
3. Bayoumi, S. (1992). Bacteriostatic Effect of some spices and their utilization in the manufacture of yoghurt. Chemie- Microbiologie -Technologie-der- Leben Smittel, 14(1/2): 21-26.
4. Baytop T. (1984). Türkiye'de Bitkiler ile Tedavi. İ.Ü. Yay. No: 3255, Eczacılık Fak. No: 40, İstanbul.
5. Bisht VK; Negi J S; Bhandari A K; Sundriyal RC. (2011) *Amomum subulatum* Roxb: Traditional, phytochemical and biological activities-An overview. Afr J Agric Res., 6: 5386-5390.
6. Choi EY; Park SY; Cho YO. (2011). Freeze-dried instant coffee can promote the activities of antioxidant enzymes and induce weight loss but also aggravate the plasma cholesterol profile in rats. Nutrition, 27: 1202-1205.
7. Clifford MN; Knight S. (2004). The cinnamoyl-amino acid conjugates of green robusta coffee beans. Food Chem., 87: 457–463.
8. Çon AH; Ayar A; Gökalp HY. (1998). Bazıbaharat uçucu yağlarının çeşitli bakterilere karşı antimikrobiyal etkisi. Gıda Derg., 23: 171-175.
9. Daglia M.; Papetti A.; Dacarro C.; Gazzani G. (1998). Isolation of an antibacterial component from roasted coffee. J. Pharm. Biomed. Anal. 18, 219-225.
10. Duda-Chodak, A.; Tarko, T. and Statek, M (2008). The effect of antioxidants on *lactobacillus casei* cultures. Acta Sci. Pol., Technol. Aliment. 7(4), 39-51.
11. Duke, J. A ; Bogenschutz-Godwin M.J.; DuCelliar, J. and Duke, P. K. (2002) : Hand Book of Medicinal Herbs (second ed.), CRC Press, Boca Raton pp. 153–154.
12. El-yamani MA. (2011). Cinnamon, cardamom and ginger impact as evaluated on hyperglycemic rats. J. of Specific Education 20: 664-679.
13. Eskelinen MH; Ngandu T; Tuomilehto J; Soininen H; Kivipelto M. (2009). Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study. J Alzheimers Dis., 16: 85-91.
14. Fardiaz S. (1995). Antimicrobial Activity of Coffee *(Coffea robusta)* Extract. ASEAN "Food Journal,10 (3): 31
15. Fooks, L.J.; Fuller, R.; Gibson, G.R. (1999). Prebiotics, probiotics and human gut microbiology. *International Dairy Journal* 9:53-61.
16. Freshney RI. (2000). Culture of Animal Cells: A Manual of Basic Technique. New York, USA: John Wiley & Sons Inc.
17. Fuller, Roy. (2007). History and development of probiotics, http://www.albertcllasic.net/probiotics.php.
18. Gilania A.; Qaiser b J.; Arif-ullah K. and

Jabbar Sh.A. (2007). Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom Journal of Ethnopharmacology115,(3) : pp 463-472 .
19. Hostettmann K; Marston A; Ndjoko K; Wolfender JL (2000). The Potential of African plants as a source of drugs. Current Organic Chemistry 4: 973–1010.
20. Hu G; Bidel S; Jousilahti P; Antikainen R; Tuomilehto J. (2007). Coffee and tea consumption and the risk of Parkinson's disease. Mov Disord., 22: 2242-2248.
21. Hughes, R.D.; McFarlane, I., Thabrew, M.I. G (1997). Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. J. Pharm. Pharmacol., 49: 1132-5.
22. Ilangantileke, S.; Karunaratne, C. and Maline, M. (1993). Effects of chemical pretreatment and drying temperatures on the commercial quality of cardamom (Elettaria cardamomum). Journal of Food Quality, 16 (6): 451–470.
23. Jamal, A.; Javed, K.; Asian, M. and Jafri, M.(2006). Gastro protective effect of cardamom, *Elettaria cardamomum* maton. fruits in rats. J. Ethnopharmacol, 103 (2): 149-153
24. Khan N.A. and Rahman, S.Z. (1992). The screening of Majoon-e-Azaraqi for cardiovascular and peripheral activity. Hamdard Medicus 35: 102–109.
25. Kuriyama S; Shimazu T; Ohmori K; Kikuchi N; Nakaya N, *et al.* (2006) Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA 296: 1255-1265.
26. Naidu, A. S.; Bidlack, W. R. and Clemens, R. A. (1999). Probiotic spectra of lactic acid bacteria (LAB). Critical Reviews in Food Science and Nutrition 39(1):13-126.
27. Nardini, M.; Cirillo, E.; Natella, F.; Scaccini, C. (2002). Absorption of phenolic acids in humans after coffee consumption. J Agric Food Chem., 50: 5735–5741.
28. Ogunleye, AA.; Xue, F.; Michels, KB. (2010). Green tea consumption and breast cancer risk or recurrence: a meta-analysis. Breast Cancer Res Treat 119: 477- 484.
29. Padmakumari Amma KP.; Rani MP.; Sasidharan I.; Premakumari Nisha VN. (2010). Chemical Composition, Flavonoid - Phenolic Contents and Radical Scavenging Activity of Four Major Varieties of Cardamom. Int J Biol Med Res., 1: 20-24.
30. Sarabi-Jamab, 1M. and Niazmand R. (2009). Effect of Essential Oil of *Mentha piperita* and *Ziziphora clinopodioides* on *Lactobacillus acidophilus* Activity as Bioyogurt Starter Culture. American-Eurasian J. Agric. & Environ. Sci., 6 (2): 129-131
31. Sema AĞAOĞLU; Nursel DOSTBİL; Süleyman ALEMDAR (2005). Antimicrobial Effect of Seed Extract of Cardamom (*Elettarıa cardamomum* Maton). YYÜ Vet Fak Derg, 16 (2):99-101
32. Sunnetha WJ.; Krishnakantha TP (2005) Cardamom extract as inhibitor of human platelet aggregation. Phytother Res., 19: 437-440.
33. Thielecke F, Boschmann M (2009). The potential role of green tea catechins in the prevention of the metabolic syndrome - a review. Phytochemistry 70: 11-24.
34. Verma SK.; Rajeevan V.; Bordia A.; Jain V. (2010). Greater cardamom (*Amomum subulatum* Roxb.) – A cardio-adaptogen against physical stress. J Herb Med Toxicol., 4: 55-58.

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