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Regular paper

In vitro pharmacological interaction of caffeine and first-line antibiotics is antagonistic against clinically important bacterial pathogens

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The *in vitro* antibacterial activity of pure caffeine powder and its interaction with first line antibiotic against bacterial isolates were investigated with the macrobroth dilution and the checkerboard assay methods. This study showed that caffeine and the antibiotics exhibited various degrees of antibacterial activities. While caffeine had MICs ranging between 67.19 and 268.75 µg/ml, chloramphenicol was characterized by MICs between 0.98 and 31.25 µg/ml, kanamycin — 15.63–62.5 µg/ml, nalidixic acid — 0.49–250 µg/ml, erythromycin — 0.49–62.5 µg/ml, tetracycline — 1.99–62.5 µg/ml and metronidazole — 15.63–31.25 µg/ml. Combining ½ MICs and MICs of caffeine with the antibiotics as well as direct combination of caffeine and the antibiotics resulted in significant reduction of antibiotics' effectiveness. The fractional inhibitory concentration index (FICI) for the combination of ½ MICs of caffeine with different antibiotics showed antagonistic interactions with the antibiotics except kanamycin which had additive and indifferent interactions with caffeine. The FICI of the MICs of caffeine combined with antibiotics showed a reduction in the number of antagonistic interactions as chloramphenicol, nalidixic acid and erythromycin showed some indifferent interactions while kanamycin was the only antibiotic that showed indifferent interaction against all the bacterial isolates. The direct combination of caffeine and the antibiotics resulted in significant antagonistic interactions higher than in the case when caffeine, at the ½ MICs and MICs, was combined with the antibiotics. Although caffeine demonstrated significant antibacterial activity against the selected bacterial isolates, its combination with the selected antibiotics resulted in significant antagonistic interactions. Caffeine should not be combined with antibiotics as this could result in serious therapeutic failure and, possibly, drug toxicity *in vivo*.

Key words: antibacterial activity, bacterial isolates, caffeine, antibiotics, antagonistic effects

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Abbreviations: FIC, fractional inhibitory concentration; MICs, minimum inhibitory concentrations

INTRODUCTION

Caffeine, 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione (Yen *et al.,* 2005), is a white powdered, water soluble plant alkaloid found in many plant species. This psychoactive, widely consumed as a stimulant (Belitz *et al.,* 2009; Mohanpuria *et al.,* 2010), is a purine alkaloid present in almost 100 plant species (Ashihara, 2006). Natural sources of caffeine include cocoa (DeVries *et al.*, 1981), coffee (Minamisawa *et al.,* 2004) and tea (Najafi *et al.,* 2004). The caffeine content in fresh coffee ranged between 314 and 646 mg/l (Rodrigues *et al.,* 2007) and the content for brewed tea including black tea (30.97 mg/g), green tea (18.70 mg/g) and oolong tea (23.89 mg/g) was reported by Guo and coworkers (2011), whereas caffeine content in cocoa powder was between 0.66 and 0.71 mg/g (Li *et al.,* 2012). Caffeine, a white odorless powder with a bitter taste, has a molecular weight of 194.191 g/mol, a density of 1.2 g/cm^3 and pH of 6.9. The solubility of caffeine in water is about 21.7 mg/ml at 25°C, 180 mg/ml at 80°C and 670 mg/ml at 100°C. Its boiling point is at 178°C and its melting point is at 238°C (Florey *et al.,* 1989; Agyemang-Yeboah *et al.,* 2013).

Caffeine has long been known to have numerous effects on human health (Cano-Marquina *et al.,* 2013). In humans, it is mainly metabolized into paraxanthine (84%), theobromine (12%) and theophylline (4%) (Lelo *et al.,* 1986; Etherton & Kochar, 1993). The daily average intake of caffeine through coffee consumption in the US is between 165 and 200 mg/day (Mitchell *et al.,* 2014), with adults taking about 2.4 mg per kg per day and children aged between 5 and 18 years old taking 1.1 mg per kg per day (Chou *et al.*, 1992; Desbrow *et al.,* 2007), while its daily intake in about four to nine cups of coffee is equivalent to 500 to 600 mg. This is recognized as an abuse posing serious health risks known as caffeinism which could result in caffeine dependency, headaches, restlessness, heart palpitations, nervousness, insomnia, vomiting, nausea and diarrhea (James *et al.,* 1985), stimulation of the central nervous system (Nehlig *et al.,* 1992) as well as the release of the stress hormones. When these hormones are chronically elevated, mental concentration is short-lived and fine motor coordination is impaired (Jacobson & Thurman-Lacey, 1992), the immune system is suppressed, digestion and elimination are impaired and body's normal repair mechanisms are inhibited, thereby accelerating aging process (Hill, 1991; Raber, 1998; Leonard, 2000). When stimulatory effects of caffeine are reduced significantly, there is tolerance adaptation. Due to adaptive reactions to caffeine, individuals become more sensitized to adenosine. A reduction in caffeine consumption effectively increases the normal functional effects of adenosine. This could result in undesirable withdrawal indications in tolerant users. Withdrawal symptoms may appear within 12 to 24 h after termination of caffeine intake. They may last for

a maximum of five days, which is the sufficient time, required for the adenosine receptors in the brain to revert to normal levels while mental awareness and general body coordination are restored.

Pharmacologically, caffeine inhibits muscle contraction (Youn *et al.,* 1991), acts as the antagonist of the adenosine receptors to increase their plasmatic concentration (Conlay *et al.,* 1997), potentiates the lethal effects of ionizing radiation which is important during the treatment of cancer (Sakurai *et al.,* 1999), alters glucose metabolism (Greer *et al.,* 2001), sensitizes calcium liberation channels and inhibits phosphodiesterase enzymes (Daly, 2007). Pure caffeine has antibacterial effect (Ramanaviciene *et al.,* 2003a; Cogo *et al.,* 2008; Mohammed & Al-Bayati, 2009), decreases the adherence of *Streptococcus mutants* to dental surface (Namboodiripad *et al*., 2007) and acts as the first dietary component able to protect one against Alzheimer's disease, Parkinson's disease and Huntington's disease (Ross *et al.,* 2000; Lindsay *et al.,* 2002; Ribeiro & Sebastiao, 2010). It increases the concentration of some immunocompetent cells and reinforces the activity of lysozyme to boost immunity against bacterial pathogens (Ramanaviciene *et al.,* 2002; Ramanaviciene *et al.,* 2003b; Vinod & Rangari, 2004). Although there are interactions between A2A receptors and the dopaminergic system in the brain, as adenosine hinders dopaminergic neurotransmission, the inhibition of A2A receptors by caffeine may accelerate dopaminergic activity and worsen psychotic symptoms (Kruger *et al.,* 1996) and as one of the 10 most frequently administered drugs in neonatal intensive care, caffeine is used to correct irregular heartbeat and treat apnea in premature newborns (Funk *et al.,* 2009).

There are 82 known drug interactions with caffeine (Prescription drugs/caffeine interactions, 2016. http://www.caffeineinformer.com/caffeine-drug-interactions. Accessed 01-05-2016). Caffeine increases the activity of some pain killers such as acetaminophen, tylenol and aspirin. It enhances their effectiveness and brings quicker relief by allowing rapid absorption of these drugs into the body. As a result, many over-the-counter headache drugs contain caffeine in their formula. Its combination with ergotamine in the treatment of migraine and headache clusters helps overcome the drowsiness caused by antihistamines (Nehlig *et al.,* 2000). While caffeine works synergistically with some pain relievers (Hidron *et al.,* 2007), it hinders the proper functioning of other drugs like fluvoxamine, which impedes the action of liver enzyme responsible for the breakdown of caffeine and, thus, increases the central effects and blood concentration of caffeine 5 times (Hidron *et al.,* 2007). Combining caffeine and ephedrine, which are both stimulant drugs, could result in side effects and heart problems. Taking antibiotics along with caffeine can increase the risk of having side effects (Coso *et al.,* 2012), fluoroquinolones decrease the rate of caffeine breakdown by the organism (Hidron *et al.,* 2007). As there is the dearth of information on the *in vitro* antibacterial action of pure caffeine powder and its interaction with first line antibiotics, this study investigated the effects of pure caffeine in the form of powder on the antibacterial activity of some first line antibiotics against bacterial isolates.

MATERIALS AND METHODS

Test drugs. The pure powder of caffeine was obtained from the Mangosuthu University of Technology, Durban, South Africa while the bacterial isolates and the pure powder of metronidazole, tetracycline, kanamycin, nalidixic acid, chloramphenicol and erythromycin were obtained from the University of Fort Hare, Alice, South Africa.

Test organisms. The organisms used in the study were obtained from the University of Fort Hare, Alice, South Africa and included *Pseudomonas aeruginosa* ATCC 15442*, Proteus vulgaris* CSIR 0030*, Escherichia coli* ATCC 8739*, Bacillus cereus* ATCC 10702*, Shigella sonnei* ATCC 29930, *Plesiomonas shigellosis* ATCC 379003, *Klebsiella pneumoniae* ATCC 10031*, Pseudomonas aeruginosa* ATCC 19582, *Enterococcus faecalis* ATCC 29212 and *Enterobacter cloacae* ATCC 13047.

Preparation of the samples. The antibiotics were prepared according to manufacturers' specification. Stock solutions of each of the drugs: kanamycin, tetracycline, chloramphenicol, nalidixic acid, erythromycin and caffeine, were prepared by dissolving each drug in its respective solvent.

Determination of the minimum inhibitory concentrations (MICs). The minimum inhibitory concentrations (MICs) of the caffeine and those of each of the antibiotics were determined by macrobroth dilution bioassay (Olajuyigbe & Afolayan, 2012). Overnight cultures were diluted with sterile nutrient broth. To determine the MICs of each of the drugs alone, one milliliter of stock solution of caffeine was serially diluted in sterile double strength Mueller Hinton broth in test tubes to obtain concentrations ranging between 1.05 and 537.5 µg/ml while concentrations ranging between 0.488 and 250 µg/ml were prepared for all the antibiotics. To determine the influence of the minimum inhibitory concentrations of caffeine on the antibacterial activities of the antibiotics, different concentrations of each of the antibiotics were serially prepared in double strength Mueller Hinton broth while the minimum inhibitory concentration (MICs) and half MICs ($^1/_2$ MICs) of caffeine were added to respective tubes containing different concentrations of each antibiotic. For the combination of caffeine and the antibiotics, equal volumes of stock solutions of caffeine and each antibiotic were combined before being serially diluted to concentrations corresponding to the MICs of each drug in double strength Mueller Hinton broth. After the drug preparations were serially diluted, 100 µl of each diluted bacterial culture was aseptically dispensed to the test tubes containing caffeine alone, antibiotics alone and their combinations and incubated at 37°C for 24 h. Bacterial growth was indicated by the turbidity of the test tube. The MIC values were recorded as the lowest concentration of each antibiotic showing no visible growth.

Checkerboard assay. The interactions between caffeine and antibiotics were determined using the checkerboard assay. The drug concentrations used in the checkerboard assay covered the MIC for each drug used in the assay. The fractional inhibitory concentration (FIC) was derived from the lowest concentration of the caffeine and the antibiotic used in combination and resulting in no visible growth of the test organisms in the Mueller-Hinton broth after incubation for 24 h at 37°C (Mandal *et al.,* 2004). FIC indices (FICI) were calculated using the formula: FIC index = (MIC of caffeine in combination/MIC of caffeine alone)+(MIC of antibiotic in combination/MIC of antibiotic alone). In antimicrobial combination, Eliopoulos and Eliopoulos and coworkers)1988) and Petersen *et al.,* (2006) defined synergy as $\Sigma FIC \leq 0.5$, additivity as $0.5 < \Sigma FIC \leq 1$, indifference as $1 < \Sigma FIC \leq 4$ and antagonism as $\Sigma FIC > 4$. This implies that synergy

Table 1. Minimum inhibitory concentrations of caffeine and of different antibiotics used alone

Organism	Caffeine	CHL	KAN	NAL	ERY	TET	MET
				µg/ml			
Pseudomonas aeruginosa ATCC 15442	268.75	0.98	15.63	31.25	0.49	31.25	31.25
Proteus vulgaris CSIR 0030	268.75	0.98	15.63	250	0 .49	1.99	31.25
Escherichia coli ATCC 8739	268.75	3.91	62.5	62.5	62.5	7.81	31.25
Bacillus cereus ATCC 10702	268.75	0.98	15.63	62.5	0.49	3.91	31.25
Shigella sonnei ATCC 29930	268.75	7.81	62.5	62.5	0.49	31.25	31.25
Plesiomonas shigellosis ATCC 379003	134.38	7.81	62.5	62.5	31.25	15.63	31.25
Klebsiella pneumoniae ATCC 10031	268.75	7.81	31.25	125	0.49	31.25	31.25
Pseudomonas aeruginosa ATCC 19582	134.38	31.25	62.5	62.5	62.5	62.5	31.25
Enterococcus faecalis ATCC 29212	268.75	125	31.25	62.5	0.49	7.81	31.25
Enterobacter cloacae ATCC 13047	67.19	3.91	15.63	0.49	0.49	62.5	15.63

Abreviations: CHL, Chloramphenicol; KAN, Kanamycin; NAL, Nalidixic acid; ERY, Erythromycin; TET, Tetracycline; MET, Metronidazole

determined by the checkerboard method may be defined as FIC ≤ 0.5 or FIC ≤ 1.

RESULTS

In this study, we determined the antibacterial activity of caffeine and first line antibiotics as well as the influence of caffeine on the antibacterial activity of the antibiotics. Table 1 shows the minimum inhibitory concentrations for caffeine and antibiotics used alone against the selected bacterial isolates. The caffeine and the antibiotics exhibited various degree of antibacterial activity. Caffeine had MICs ranging between 67.19 and 268.75 µg/ml, chloramphenicol had MICs of between 0.98 and 31.25 µg/ml, kanamycin – 15.63–62.5 µg/ml, nalidixic acid – 0.49–250 µg/ml, erythromycin – 0.49–62.5 µg/ml, tetracycline – 1.99–62.5 µg/ml and metronidazole – 15.63–31.25 µg/ml. At 268.75 µg/ml, caffeine inhibited the growth of the bacterial isolates except *Pl. shigellosis* ATCC 379003 and *Ps. aeruginosa* ATCC 15442 which were inhibited at 134.38 µg/ml and *E. cloacae* ATCC 13047 which was inhibited at a concentration of 67.19 µg/ml. At 0.98 µg/ml, chloramphenicol inhibited *Ps. aeruginosa* ATCC 15442, *P. vulgaris* CSIR 0030 and *B. cereus* ATCC 10702. At 3.91 µg/ml, *E. coli* ATCC 8739 and *E. cloacae* ATCC 13047 were inhibited. At 7.81 µg/ml, *S. sonnei* ATCC 29930, *Pl. shigellosis* ATCC 379003 and *K. pneumoniae* ATCC 10031 were inhibited. At 31.25 µg/ml, the drug inhibited the growth of *Ps. aeruginosa* ATCC 15442 and *E. faecalis* was inhibited at 125 µg/ml. Kanamycin, on the other hand, inhibited *Ps. aeruginosa* ATCC 19582, *P. vulgaris* CSIR 0030, *E. cloacae* and *B. cereus* ATCC 10702 at a concentration of 15.63 µg/ml. At 62.5 µg/ml, *E. coli* ATCC 8739, *S. sonnei* ATCC 29930, *Pl. shigellosis* ATCC 379003 and *Ps. aeruginosa* ATCC 15442 were inhibited. At 31.25 µg/ml, *E. faecalis* and *K. pneumoniae* ATCC 10031 were inhibited. Nalidixic acid inhibited the growth of most organisms at 62.5 µg/ml except *Ps. aeruginosa* ATCC 19582 inhibited at 31.25µg/ml, *P. vulgaris* CSIR 0030 at 250 µg/ml, *K. pneumoniae* ATCC 10031 at 125 µg/ml and *E. cloacae* ATCC 13047 at 0.49 µg/ml. Erythromycin inhibited the growth of most of the organisms at 0.49 µg/ml but *Ps. aeruginosa* ATCC 15442 and *E. coli* ATCC 8739 were inhibited at 62.5 µg/ml and *Pl. shigellosis* ATCC 379003 was inhibited at 31.25 µg/ml. Tetracycline inhibited the growth of *Ps. aeruginosa* ATCC 19582, *S. sonnei* ATCC 29930, *K. pneumoniae* ATCC 10031 at 31.25 µg/ml, *Ps. aeruginosa* ATCC 15442 and *E. cloacae* ATCC 13047 were inhibited at 62.5 µg/ml, *E. faecalis* ATCC 29212 and *E. coli* ATCC 8739 was inhibited at 7.8 µg/ml, *Pl. shigellosis* ATCC 379003 was inhibited at 15.63 µg/ml, *P. vulgaris* CSIR 0030 was inhibited at 1.99 µg/ml and *B. cereus* ATCC 10702 was inhibited at 3.91 µg/ml. Metronidazole inhibited all of the bacterial isolates at 31.25 µg/ml except *E. cloacae* being inhibited at 15.63 µg/ml.

Table 2 shows the influence of caffeine at half of the minimum inhibitory concentrations ($^1/_2$ MICs) on the antibacterial activity of the antibiotics. Combining caffeine with the antibiotics at its half MICs resulted in significant increase of the MICs of the antibiotics. Chloramphenicol that inhibited the bacterial isolates at concentrations ranging between 0.98 and 125 µg/ml when used alone had MICs ranging between 3.91 and 250 µg/ml when used in combination with caffeine at its half MICs. At 31.25 µg/ml, this antibiotic inhibited the growth of *P. vulgaris* CSIR 0030 and *E. coli* ATCC 8739. However, *Ps. aeruginosa* ATCC 19582 and *B. cereus* ATCC 10702 were inhibited at 3.91 µg/ml, *S. sonnei* ATCC 29930, *K. pneumonia* ATCC 10031, *Ps. aeruginosa* ATCC 15442 and *E. faecalis* ATCC 29212 at 250 µg/ml while *P. shigellosis* ATCC 379003 and *E. cloacae* were inhibited at 125 µg/ml.

Kanamycin having MICs ranging between 15.63 and 62.5 µg/ml when used alone had its MICs ranging between 1.95 and 62.5 µg/ml when combined with caffeine at its ½ MICs. It inhibited the growth of *P. vulgaris* CSIR 0030 and *E. coli* ATCC 8739 at 1.95 µg/ml, *K. pneumoniae* ATCC 10031 and *E. faecalis* ATCC 29212 at 15.63 µg/ml, *P. shigellosis* ATCC 379003 and *E. cloacae* ATCC 13047 were inhibited at 31.25 µg/ml while *Ps. aeruginosa* ATCC 15442 and *Ps. aeruginosa* ATCC 19582 were inhibited at 62.5 µg/ml. While nalidixic acid used alone had MICs ranging between 0.49 and 250 µg/ml, its combination with caffeine ($^1/_2$ MICs) resulted in its inhibitory concentrations, effective against all the test bacterial isolates, being greater than 250 µg/ml. On the other hand, erythromycin had MICs of 0.49 to 62.5 µg/ml when used alone with most of the isolates being inhibited at 0.49 µg/ml and it had its inhibitory

Table 2. Caffeine at half of its minimum inhibitory concentrations ($^1/_2$MIC) in combination with the different antibiotics

Organism	CHL/CAF	KAN/CAF	NAL/CAF	ERY/CAF	TET/CAF	MET/CAF
			µg/ml.			
Pseudomonas aeruginosa ATCC 15442	3.91/134.8	62.5/134.8	>250/134.8	62.5/134.8	15.63/134.8	125/134.8
Proteus vulgaris CSIR 0030	31.25/134.8	1.95/134.8	>250134.8	62.5/134.8	7.81/134.8	125/134.8
Escherichia coli ATCC 8739	31.25/134.8	1.95/134.8	>250/134.8	125/134.8	15.63/134.8	250/134.8
Bacillus cereus ATCC 10702	3.91/134.8	7.81/134.8	>250/134.8	62.5/134.8	15.63/134.8	62.5/134.8
Shigella sonnei ATCC 29930	250/134.8	7.81/134.8	>250/134.8	62.5/134.8	125/134.8	125/134.8
Plesiomonas shigellosis ATCC 379003	125/134.8	31.25/134.8	>250/134.8	125/134.8	62.5/134.8	125/134.8
Klebsiella pneumoniae ATCC 10031	250/134.8	15.63/134.8	>250/134.8	125/134.8	125/134.8	125/134.8
Pseudomonas aeruginosa ATCC 19582	250/67.4	62.5/67.4	>250/67.4	125/67.4	62.5/67.4	125/67.4
Enterococcus faecalis ATCC 29212	250/134.8	15.63/134.8	>250/134.8	250/134.8=	125/134.8	62.5/134.8
Enterobacter cloacae ATCC 13047	125/37.4	31.25/37.4	>250/37.4	125/37.4	62.5/37.4	62.5/37.4

Abbreviations: The combination of caffeine and antibiotics. CHL, Chloramphenicol; KAN, Kanamycin; NAL, Nalidixic acid; ERY, Erythromycin; TET, Tetracycline; MET, Metronidazole; CAF, Caffeine

Table 3. Minimum inhibitory concentrations (MICs) of caffeine in combination with different antibiotics

Organism	CHL/CAF	KAN/CAF	NAL/CAF	ERY/CAF	TET/CAF	MET/CAF
			µg/ml			
Pseudomonas aeruginosa ATCC 15442	31.25/268.75	0.98/268.75	250/268.75	7.81/268.75	7.81/268.75	250/268.75
Proteus vulgaris CSIR 0030	15.63/268.75	1.95/268.75	<250/268.75	125/268.75	15.63/268.75	250/268.75
Escherichia coli ATCC 8739	62.5/268.75	62.5/268.75	<250/268.75	125/268.75	31.25/268.75	250/268.75
Bacillus cereus ATCC 10702	62.5/268.75	31.25/268.75	250/268.75	<250/268.75	31.25/268.75	<250/268.75
Shigella sonnei ATCC 29930	125/268.75	31.25/268.75	250/268.75	<250/268.75	250/268.75	<250/268.75
Plesiomonas shigellosis ATCC 379003	31.25/134.38	125/134.38	125/134.38	250/134.38	7.81/134.38	250/134.38
Klebsiella pneumoniae ATCC 10031	125/268.75	62.5/268.75	250/268.75	125/268.75	125/268.75	250/268.75
Pseudomonas aeruginosa ATCC 19582	62.5/134.38	31.25/134.38	250/134.38	250/134.38	62.5/134.38	250/134.38
Enterococcus faecalis ATCC 29212	125/268.75	62.5/268.75	250/268.75	250/268.75	250/268.75	250/268.75
Enterobacter cloacae ATCC 13047	31.25/67.19	7.81/67.19	<250/67.19	250/67.19	125/67.19	125/67.19

Abbreviations: CHL, Chloramphenicol; KAN, Kanamycin; MET, Metronidazole; TET, Tetracycline; NAL, Nalidixic acid; ERY, Erythromycin

Table 4. Antibacterial effects of direct combination of caffeine and the different antibiotics against bacterial isolates

Organism	CHL/CAF	KAN/CAF	NAL/CAF	ERY/CAF	TET/CAF	MET/CAF
			µg/ml			
Pseudomonas aeruginosa ATCC 15442	125/125	250/250	250/250	7.81/7.81	250/250	250/250
Proteus vulgaris CSIR 0030	62.5/62.5	250/250	125/125	62.5/62.5	125/125	250/250
Escherichia coli ATCC 8739	125/125	125/125	125/125	125/125	125/125	250/250
Bacillus cereus ATCC 10702	125/125	125/125	250/250	250/250	250/250	250/250
Shigella sonnei ATCC 29930	125/125	125/125	250/250	250/250	250/250	250/250
Plesiomonas shigellosis ATCC 379003	125/125	125/125	250/250	250/250	125/125	250/250
Klebsiella pneumoniae ATCC 10031	125/125	125/125	250/250	250/250	250/250	250/250
Pseudomonas aeruginosa ATCC 19582	125/125	125/125	250/250	250/250	125/125	250/250
Enterococcus faecalis ATCC 29212	125/125	250/250	250/250	250/250	125/125	250/250
Enterobacter cloacae ATCC 13047	125/125	125/125	250/250	250/250	250/250	250/250

Abbreviations: CHL, Chloramphenicol; KAN, Kanamycin; MET, Metronidazole; TET, Tetracycline; NAL, Nalidixic acid; ERY, Erythromycin

Table 5. Fractional inhibitory concentrations index of the effects of half of the minimum inhibitory concentrations of caffeine ($^1/_2$MICs) on the antibacterial activity of the different antibiotics

Organism	CHL/ CAF	Rem	KAN/ CAF	Rem	NAL/ CAF	Rem	ERY/ CAF	Rem	TET/ CAF	Rem	MET/ CAF	Rem
	(FICI)		(FICI)		(FICI)		(FICI)		(FICI)		(FICI)	
Pseudomonas aeruginosa ATCC 15442	4.5	Antag	4.5	Antag	8.5	Antag	128.5	Antag	1	Add	4.5	Antag
Proteus vulgaris CSIR 0030	32.5	Antag	0.63	Add	4.5	Antag	128.5 A	Antag	4.5	Antag	4.5	Antag
Escherichia coli ATCC 8739	8.5	Antag	0.53	Add	4.5	Antag	2.5	Indiff	2.5	Indiff	8.5	Antag
Bacillus cereus ATCC 10702	4.5	Antag	1.0	Add	4.5	Antag	128.5	Antag	4.5	Antag	2.5	Indiff
Shigella sonnei ATCC 29930	32.5	Antag	0.75	Add	4.5	Antag	128.5	Antag	4.5	Antag	4.5	Antag
Plesiomonas shigellosis ATCC 379003	16.5	Antag	1.0	Add	4.5	Antag	4.5	Antag	4.5	Antag	4.5	Antag
Klebsiella pneumoniae ATCC 10031	32.5	Antag	1.0	Add	1.5	Indiff	255.5	Antag	4.5	Antag	4.5	Antag
Pseudomonas aeruginosa ATCC 19582	8.5	Antag	1.5	Indiff	4.5	Antag	2.5	Indiff	1.5	Indiff	4.5	Antag
Enterococcus faecalis ATCC 29212	2.5	Indiff	1.0	Add	4.5	Antag	500.5	Antag	8.5	Antag	2.5	Indiff
Enterobacter cloacae ATCC 13047	32.5	Antag	2.5	Indiff	10.5	Antag	255.5	Antag	1.5	Indiff	4.5	Antag

Abbreviations: Add, Additive; Antag, Antagonistic; Indiff, Indifferent; FICI, Fractional inhibitory concentration index; Chl, Chloramphenicol; Kan, Kanamycin; Nal, Nalidixic acid; Ery, Erythromycin; Tet, Tetracycline; Met, Metronidazole; CAF, Caffeine; Rem, Remarks

concentrations increased to 62.5–250 μg/ml. Used in the combination, erythromycin inhibited *Ps. aeruginosa* ATCC 19582, *P. vulgaris* CSIR 0030, *B. cereus* ATCC 10702 and *S. sonnei* ATCC 29930 at 62.5 μg/ml, *E. coli* ATCC 8739, *P. shigellosis* ATCC 379003, *K. pneumoniae* ATCC 10031, *Ps. aeruginosa* ATCC 15442 and *E. cloacae* at 125 μg/ml and *E. faecalis* ATCC 29212 at 250 μg/ml. Half MICs of caffeine increased the MICs of tetracycline from between 1.99 and 62.5 μg/ml when used alone to 7.81–125 μg/ml when in combination. In the combination with caffeine, tetracycline inhibited *P. vulgaris* CSIR 0030 at 7.81 μg/ml, *Ps. aeruginosa* ATCC 19582, *E. coli* ATCC 8739 and *B. cereus* ATCC 10702 at 15.63 μg/ml, *P. shigellosis* ATCC 379003, *Ps. aeruginosa* ATCC 15442 and *E. cloacae* at 62.5 μg/ml while *K. pneumoniae* ATCC 10031 and *E. faecalis* ATCC 29212 were inhibited at 125 μg/ml. Although the MICs of metronidazole used alone ranged from 15.63 to 31.25 μg/ml, they increased to between

Table 6. Fractional inhibitory concentration index of the effect of minimum inhibitory concentrations (MICs) of caffeine on the antibacterial activities of different antibiotics

Organism	CHL/ CAF	Rem	KAN/ CAF	Rem	NAL/ CAF	Rem	ERY/ CAF	Rem	TET/ CAF	Rem	MET/ CAF	Rem
	(FICI)		(FICI)		(FICI)		(FICI)		(FICI)		(FICI)	
Pseudomonas aeruginosa ATCC 15442	33	Antag	1.06	Indiff	9	Antag	17	Antag	1.25	Indiff	9	Antag
Proteus vulgaris CSIR 0030	17	Antag	1.13	Indiff	2	Indiff	256	Antag	9	Antag	9	Antag
Escherichia coli ATCC 8739	17	Antag	2	Indiff	5	Antag	3	Indiff	5	Antag	9	Antag
Bacillus cereus ATCC 10702	65	Antag	3	Indiff	5	Antag	511	Antag	9	Antag	9	Antag
Shigella sonnei ATCC 29930	17	Antag	1.5	Indiff	5	Antag	511	Antag	9	Antag	9	Antag
Plesiomonas shigellosis ATCC 379003	5	Antag	3	Indiff	3	Indiff	9	Antag	1.5	Indiff	9	Antag
Klebsiella pneumoniae ATCC 10031	17	Antag	3	Indiff	3	Indiff	256	Antag	5	Antag	9	Antag
Pseudomonas aeruginosa ATCC 19582	3	Indiff	1.5	Indiff	5	Antag	3	Indiff	2	Indiff	9	Antag
Enterococcus faecalis ATCC 29212	2	Indiff	26	Antag	5	Antag	511	Antag	32	Antag	9	Antag
Enterobacter cloacae ATCC 13047	9	Antag	1.5	Indiff	511	Antag	511	Antag	3	Indiff	9	Antag

Abbreviations: Add, Additive; Antag, Antagonistic; Indiff, Indifferent; FICI, Fractional inhibitory concentration index; Chl, Chloramphenicol; Kan, Kanamycin; Nal, Nalidixic acid; Ery, Erythromycin; Tet, Tetracycline; Met, Metronidazole, CAF, Caffeine, Rem, Remarks

Table 7. Fractional inhibitory concentration index of the effect of direct combination of caffeine and the different antibiotics against the bacterial isolates

Organism	CHL/CAF	Rem	KAN/CAF	Rem	NAL/CAF	Rem	ERY/CAF	Rem	TET/CAF	Rem	MET/CAF	Rem
	FICI		FICI		FICI		FICI		FICI		FICI	
Pseudomonas aeruginosa ATCC 15442	129	Antag	257	Antag	256	Antag	8	Antag	256	Antag	256	Antag
Proteus vulgaris CSIR 0030	64	Antag	257	Antag	129	Antag	64	Antag	129	Antag	256	Antag
Escherichia coli ATCC 8739	33	Antag	326	Antag	33	Antag	33	Antag	33	Antag	65	Antag
Bacillus cereus ATCC 10702	128	Antag	128	Antag	256	Antag	256	Antag	256	Antag	256	Antag
Shigella sonnei ATCC 29930	17	Antag	17	Antag	33	Antag	33	Antag	33	Antag	33	Antag
Plesiomonas shigellosis ATCC 379003	17	Antag	17	Antag	34	Antag	34	Antag	33	Antag	34	Antag
Klebsiella pneumoniae ATCC 10031	17	Antag	5	Antag	33	antag	33	Antag	33	Antag	33	Antag
Pseudomonas aeruginosa ATCC 19582	5	Antag	17	Antag	10	Antag	10	Antag	5	Antag	10	Antag
Enterococcus faecalis ATCC 29212	2	Indiff	3	Indiff	2.93	Antag	2.93	Antag	1.5	Indiff	2.93	Antag
Enterobacter cloacae ATCC 13047	34	Antag	32	Antag	68	Antag	68	Antag	68	Antag	68	Antag

Abbreviations: Add, Additive; Antag, Antagonistic; Indiff, Indifferent; FICI, Fractional inhibitory concentration index; Chl, Chloramphenicol; Kan, Kanamycin; Nal, Nalidixic acid; Ery, Erythromycin; Tet, Tetracycline; Met, Metronidazole; CAF, Caffeine; Rem, Remarks

62.5 and 250 μg/ml when combined with caffeine at its half MICs. Upon the combination of metronidazole with caffeine at ½ MICs, *E. faecalis* ATCC 29212, *E. cloacae* ATCC 13047 and *B. cereus* ATCC 10702 were inhibited at 62.5 μg/ml and *E. coli* ATCC 8739 was inhibited at 250 μg/ml while other bacterial isolates were inhibited at 125 μg/ml.

Table 3 shows the influence of the MIC of the caffeine on the antibacterial activity of the antibiotics. In combinations with caffeine, chloramphenicol had MICs ranging between 15.63 and 125 μg/ml. It inhibited *P. vulgaris* CSIR 0030 at 15.63 μg/ml, *E. cloacae, Ps. aeruginosa* ATCC 19582 and *P. shigellosis* ATCC 379003 at 31.25 μg/ml, *E. coli, B. cereus* ATCC 10702 and *Ps. aeruginosa* ATCC 15442 at 62.5 μg/ml, and *S. sonnei* ATCC 29930, *E. faecalis* ATCC 29212, *K. pneumoniae* ATCC 10031 at 125 μg/ml. While the interaction between caffeine and kanamycin resulted in MICs ranging between 0.98 and 62.5 μg/ml for kanamycin, it inhibited *Ps. aeruginosa* ATCC 19582 at 0.98 μg/ml, Proteus *vulgaris* CSIR 0030 at 1.95 μg/ml, *E.* cloacae at 7.81 μg/ml, *E. coli* ATCC 8739, *Ps. aeruginosa* ATCC 1542, *S. sonnei* ATCC 29930 and *B. cereus* ATCC 10702 at 31.25 μg/ml, whereas *E. faecalis* ATCC 29212 and *K. pneumoniae* ATCC 10031 at 62.5 μg/ml. For nalidixic acid, its MICs ranged between 125 and >250 μg/ml when combined with caffeine at its MICs. With the exception of *P. shigellosis* ATCC 379003 being inhibited at 125 μg/ml and *E. cloacae* ATCC 13047, *E. coli* ATCC 8739 and *P. vulgaris* CSIR 0030 being inhibited at concentrations greater than 250 μg/ml, other isolates were inhibited at 250 μg/ml when nalidixic acid was combined with MICs of caffeine. Combining erythromycin with caffeine increased the MICs up to 7.81–>250 μg/ml with most of the isolates being inhibited at 250 and >250 μg/ml. While the combination of caffeine at MICs with tetracycline resulted in tetracycline's MICs being increased to between 7.81 and 250 μg/ml, those of metronidazole was between 125 and >250 μg/ml with most of the isolates being inhibited at 250 and >250 μg/ml except *E. cloacae* ATCC 13047 being inhibited at 125 μg/ml. The direct combination of caffeine with the antibiotics resulted in significant increase in the minimum inhibitory concentrations of the antibiotics against all the bacterial isolates as shown in Table 4.

In Tables 5 to 7, the fractional inhibitory concentration index was used to find out if the interactions between caffeine and the antibiotics were antagonistic, synergistic, additive or indifferent. The fractional inhibitory concentration of the combination of ½ MICs of caffeine with the different antibiotics showed that all the antibiotics exhibited antagonistic except kanamycin having additive and indifferent interactions with caffeine as shown in Table 5. However, combining the antibiotics with the MICs of caffeine showed a reduction in number of antagonistic interactions as chloramphenicol, nalidixic acid and erythromycin showed some indifferent while kanamycin was the only antibiotic that showed indifferent interaction against all the selected bacterial strains as shown in Table 6. The direct combination of caffeine and each of the antibiotics resulted in significant antagonistic interactions with inhibitory concentrations higher than when caffeine was combined with the antibiotics at the ½ MICs and MICs as shown in Table 7.

DISCUSSION

It is a common practice to wash down drugs and orally administer medications with caffeine containing soft drinks such as cola and energy drinks. Occasionally, caffeine containing food such as cocoa, coffee and tea is used in beverages to reduce the after taste some bitter drugs would leave after an oral administration. Some of these caffeine containing foods are used to persuade little children to take bitter drugs. In pharmaceutical drugs such as ergotamine, caffeine is included for the treatment of migraine headaches and is combined with non-steroidal anti-inflammatory drugs for relieving pain (Saw-

ynok, 1995). These practices have continued for ages without exceptional consideration for possible drug-drug or drug-food interactions between the caffeine contained by the food and the different medications, especially antibiotics used in treating bacterial infections. Therefore, it becomes necessary to investigate interactions between caffeine and antibiotics (Kang *et al.,* 2012).

Although several reports showed that caffeine possesses antibacterial properties, most of these studies focused on caffeine containing food. For instance, Ramanaviciene and coworkers (2002) reported antibacterial activity of caffeine against *Ps. flourescens* and *E. coli.* Mohammed and Al-Bayati (2009) reported antibacterial activity of caffeine from *Coffee arabica* (coffee beans) and *Camelia sinensis* (green tea leaves) against *S. aureus, B. cereus, E. coli*, *P. mirabilis* and *K. pneumoniae*. Inhibitory effects of *Coffea canephora* extracts against *L. pneumophila* (Furuhata *et al.,* 2002), *S. mercescens* and *E. cloacae* (Almeida *et al.,* 2006) and *S. mutans* (Antonio *et al.,* 2010; Almeida *et al.,* 2012) were also reported while Suárez-Quiroz and coworkers (2004), Wilmot (2006) and Chen and coworkers (2013) showed that caffeine inhibits mold and fungi. However, there is the dearth of information on the antibacterial activities of pure caffeine powder. In this study, the minimum inhibitory concentration of pure caffeine powder ranged between 67.19 and 268.75 µg/ml. While most of the bacterial isolates were inhibited at 268.75 µg/ml, *P. shigellosis* ATCC 379003 and *Ps. aeruginosa* ATCC 15442 were inhibited at 134.38 µg/ml and *E. cloacae* ATCC 13047 was inhibited at 67.19 µg/ml. These results are in agreement with previous reports. Mohammed and Al-Bayati (2009) reported that MICs of caffeine coffee ranged between 62.5 and 250 µg/ml and green tea caffeine ranged from 62.5 to 500 µg/ml while Pruthviraj and coworkers (2011) reported MICs of caffeine isolated from tea between 65.5 and 250 µg/ml and green tea caffeine between 65.5 and 500 µg/ml. The activity of pure caffeine, used in this study, was further corroborated by Ramanaviciene and coworkers (2003b) who indicated that pure caffeine has a direct antibacterial effect.

Furthermore, the interactions of caffeine with antibiotics were previously reported. While Charles and Rawal (1979) indicated that caffeine decreased the antibacterial activity of chloramphenicol and tetracycline hydrochloride, Hosseinzadeh and coworkers (2006) reported that caffeine works synergistically with carbenicillin, ceftizoxime and gentamicin which are effective against *Ps. aeruginosa* and *S. aureus*. Esinome and coworkers (2008) reported that caffeine combined with ampicillin resulted in indifferent interaction while its combination with benzypenicillin was antagonistic. Kang and coworkers (2012) reported that there are no synergistic interactions between caffeine and bleomycin or cisplatin but the activities of ciprofloxacin were decreased when paired with caffeine. In this study, the combination of caffeine with chloramphenicol, nalidixic acid, erythromycin, tetracycline and metronidazole resulted, mostly, in antagonistic interactions with the exception of the interaction with kanamycin that resulted mostly in either additive or indifferent effects. Kang and coworkers (2012), however, indicated that kanamycin activity is increased upon the addition of 0.25 mg/ml (0.025% w/v) of caffeine in a previous study.

Caffeine increases the susceptibility of bacteria and higher cells to different antibiotics and DNA-damaging agents when used in pre-treatment (Grigg *et al.,* 1985; Petru *et al.* 1990; Selby and Sancar, 1990) by intercalating into the DNA (Tornaletti *et al.,* 1989). Although caffeine interacts with bacterial nucleic acids (Sacks & Thompson, 1977; Kawamukai *et al.,* 1986), inhibits DNA synthesis (Sandlie *et al.,* 1983; Osman & McCready, 1998) and cause frameshift mutations (Pons & Muller, 1990), it can also interact with the enzymes responsible for repair of bacterial DNA damage by inhibiting ATP-dependent enzymes (Selby & Sancar, 1990) and inactivate ataxia-telangiectasia-mutated (ATM) and ATM-and-Rad3-related proteins responsible for the genome stability (Cortez, 2003). While the variety of the effects of combining caffeine and these antibiotics may resulted from the influence of the physicochemical interactions between caffeine and the drugs *in vitro,* the interactions could have resulted from the formation of complexes between the caffeine and the antibiotics (Veselkov *et al.,* 2002). The complex formation with caffeine through hydrophobic van der Waals interactions (Lachman & Ravin, 1959; Matha & adbels, 1982; Kapuscinski & Kimmel 1993; Larsen *et al.,* 1996) may reduce the *in vitro* antibacterial activity of the less polar antibiotics. The antagonistic interaction or reduction in the antibacterial activities of most of these antibiotics could also be a result of caffeine and antibiotic molecules competing for the same binding sites on DNA or "intercepting" or "protecting" properties of caffeine (Traganos *et al.,* 1991; Davies *et al.,* 2001) or its ability to stimulate hydrophobic molecules into their dimeric structures (Banerjee *et al.,* 2012). Aminoglycoside antibiotics, such as kanamycin, target the ribosomes and interfere with the fidelity of protein synthesis (Poehlsgaard & Douthwaite, 2005). The interaction between caffeine and kanamycin could result from the complementary activity of both agents. Caffeine could increase susceptibility to kanamycin and intercalate into DNA (Tornaletti *et al.,* 1989) while kanamycin induces mistranslation of mRNA to protein (Misumi & Tanaka, 1980) and damages DNA base pairs (Kang *et al.,* 2012). On the other hand, caffeine could increase susceptibility to erythromycin but the putative complexes formed between caffeine and erythromycin possibly prevented both antibacterial agents from reaching their target sites of action.

CONCLUSION

In conclusion, caffeine demonstrated significant antibacterial activity against the selected bacterial isolates. However, its combination with the selected antibiotics resulted in significant antagonistic interactions against the bacterial isolates indicating that, against usual practice of using caffeine containing food or drinks in oral drug administration, caffeine should not be combined with antibiotics as this could result in serious therapeutic failure and, possibly, drug toxicity *in vivo*.

REFERENCES

Agyemang-Yeboah F, Oppong SY (2013) Caffeine: The wonder compound, chemistry and properties. Ghana: Kwame Nkrumah University, MSc thesis

Almeida AAP, Farah A, Silva DAM, Nunan EA, Gloria MBA (2006) Antibacterial activity of coffee extracts and selected coffee chemical compounds against Enterobacteria. *J Agric Food Chem* **54**: 8738–8743. doi: 10.1021/jf0617317

Almeida AAP, Naghetini CC, Santos VR, Antonio AG, Farah A, Glória MBA (2012) Influence of natural coffee compounds, coffee extracts and increased levels of caffeine on the inhibition of *Streptococcus mutans*. *Food Res Int* **49**: 459–461. doi: 10.1016/j.foodres.2012.07.026

Antonio AG, Moraes RS, Perrone D, Maria LC, Santos KRN, Iorio NLP, Farah A (2010) Species, roasting degree and decaffeination influence the antibacterial activity of coffee against *Streptococcus mutans*. *Food Chem* **118**: 782–788. doi: 10.1016/j.foodchem.2009.05.063

Ashihara H (2006) Metabolism of alkaloids in coffee plants. *Brazil J Plant Physiol* **18**: 1–8. doi: 10.1590/S1677- 04202006000100001
Banerjee S, Verma PK, Mitra RK, Basu G, Pal SK (2012) Probing the interior of self-assembled caffeine dimer at various temperatures. *J Fluoresc* **22**: 753–769. doi: 10.1007/s10895-011-1011-3
Belitz HD, Grosch W, Schieberle P (2009) Food Chemistry: Coffee, Tea, Cocoa. Berlin Heidelberg: Springer-Verlag
Cano-Marquina A, Tarín JJ, Cano A (2013) The impact of coffee on health. *Maturitas* **75**: 7–21. doi: 10.1016/j.maturitas.2013.02.002.
Cano-Marquina A, Tarin JJ, Cano A (2013) The impact of coffee on health. *Maturitas* **75**: 7–21. http://dx.doi.org/10.1016/j.maturitas.2013.02.002
Charles BG, Rawal BD (1979) The combined action of methylxanthines with erythromycin and tetracyclines on *Staphylococcus aureus*. *Microbios Lett* **10**: 143–147.
Chen, J, Zhang S, Yang X (2013) Control of brown rot on nectarines by tea polyphenol combined with tea saponin. *Crop Protection* **45**: 29–35. Doi: 10.1016/j.cropro.2012.11.006
Chou T. (1992) Wake up and smell the coffee: Caffeine, coffee and medical consequences. *Western J Med* **157**: 544–553
Cogo K, Montan MF, Bergamaschi CdeC, Andrade ED, Rosalen PL, Groppo FC (2008) *In vitro* evaluation of the effect of nicotine, cotinine and caffeine on oral microorganisms. *Can J Microbiol* **54**: 501–508. doi: 0.1139/W08-032
Conlay LA, Conant JA, DeBros F, Wurtman R (1997) Caffeine alters plasma adenosine levels. *Nature* **389**: 136. doi: 10.1038/38160
Coso JD, Munoz-Fernandez VE, Munoz G, Fernandez-Elias VE, Ortega JF, Hamouti N, Barbero JC, Munoz-Guerra J (2012) Effects of a caffeine-containing energy drink on simulated soccer performance. *PLoS ONE* **7**: e31380. doi:10.1371/journal.pone.0031380
Cortez D (2003) Caffeine inhibits checkpoint responses without inhibiting the ataxia-telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR) protein kinases. *J Biol Chem* **278**: 37139–37145. doi: 10.1074/jbc.M307088200
Daly JW (2007) Caffeine analogs: biomedical impact. *Cell Mol Life Sci* **64**: 2153–2169
Davies DB, Veselkov DA, Djimant LN, Veselkov AN (2001) Heteroassociation of caffeine and aromatic drugs and their competitive binding with a DNA oligomer. *Eur Biophys J* **30**: 354–366
Desbrow B, Hughes R, Leveritt M, Scheelings P (2007) An examination of consumer exposure to caffeine from retail coffee outlets. *Food Chem Toxicol* **45**: 1588–1592. doi:10.1016/j.fct.2007.02.020
DeVries JW, Johnson KD, Heroff JC (1981) HPLC determination of caffeine and theobromine content of various natural and red dutch cocoas. *Hoppe Seyler's Z Physiol Chem* **358**: 807–817
Eliopoulos GM, Eliopoulos CT (1988) Antibiotic combinations: should they be tested? *Clin Microbiol Rev* 1: 139-156.
Esinome CO, Okoye FBC, Nworu CS, Agubata CO (2008). *In vitro* interaction between caffeine and come penicillin antibiotics against *Staphylococcu aureus*. *Trop J Pharm Res* **7**: 969–974
Etherton GM, Kochar MS (1993) Coffee facts and controversies. *Arch of Fam Med* **2**: 317–322
Florey K (1986) Analytical profiles of drug substances. In *Analytical profiles of drug substances* Al-Badr AA, Brewer AG, Brenner SG, Deangelis JN, Mollica AJ eds. p 71. London: Academic Press
Funk GD (2009) Losing sleep over the caffeination of prematurity. *J Physiol* **587**: 5299–5300. doi: 10.1113/jphysiol.2009.182303
Furuhata K, Dogazaki C, Hara M, Furuyama M (2002) Inactivation of *Legionella pneumophila* by phenol compounds contained in coffee. *J Antibact Antifung Agents* **30**: 291–297
Greer F, Hudson R, Ross R, Graham T (2001) Caffeine ingestion decreases glucose disposal during a hyperinsulinemic – euglycemic clamp in sedentary humans. *Diabetes* **50**: 2349–2354. doi: 10.2337/diabetes.50.10.2349
Grigg GW, Hall RM, Hart NK, Kavulak DR, Lamberton JA, Lane A (1985) Amplification of the antibiotic effects of the bleomycins and tallysomycins: its dependence on the nature of the variable groups. *J Antibiotics* **38**: 99–110
Suárez-Quiroz ML, González-Rios O, Barel M, Guyot B, Schorr-Galindo S, Guiraud JP (2004) Effect of chemical and environmental factors on *Aspergillus ochraceus* growth and toxigenesis in green coffee. *Food Microbiol* **21**: 629–634
Guo S, Zhu Q, Yang B, Wang J, Ye B (2011) Determination of caffeine content in tea based on poly(safranine T) electro active film modified electrode. *Food Chem* **129**: 1311–1314. doi: 10.1016/j.foodchem.2011.05.095
Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK, National Healthcare Safety Network Team & Participating National Healthcare Safety Network Facilities (2008) NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Contr Hosp Epidemiol* **29**: 996–1011. doi: 10.1086/591861
Hill P (1991) It is not what you eat, but how you eat it digestion, lifestyle, nutrition. *Nutrition* **7**: 385–395
Hosseinzadeh H, Bazzaz BS, Sadat MM (2006) *In vitro* evaluation of methylxanthines and some antibiotics: interaction against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Iranian Biomed J* **10**: 163–167
Jacobson BH, Thurman-Lacey SR (1992) Effect of caffeine on motor performance by caffeine-naive and familiar subjects. *Perceptual and Motor Skills* **74**: 151–157
James JE, Paull I (1985) Caffeine and human reproduction. *Rev Env Health* **5**: 151–167
Kang TM, Yuan J, Nguyen A, Becket E, Yang H, Miller JH (2012) The aminoglycoside antibiotic kanamycin damages DNA bases in *Escherichia coli*: caffeine potentiates the DNA-damaging effects of kanamycin while suppressing cell killing by ciprofloxacin in escherichia coli and bacillus anthracis. *Antimicrob Agents Chemother* **56**: 3216–3223. doi: 10.1128/AAC.00066-12
Kapuscinski J, Kimmel M (1993) Thermodynamic model of mixed aggregation of intercalators with caffeine in aqueous solution. *Biophys Chem* **46**: 153–163. doi: 10.1016/0301-4622(93)85022-A
Kawamukai M, Murao K, Utsumi R, Himeno M, Komano T (1986) Cell filamentation in an *Escherichia coli* K-12 fic mutant caused by theophylline or an adenylate cyclase gene (cya)-containing plasmid. *FEMS Microb Lett* **34**: 117–120. doi: 10.1111/j.1574-6968.1986.tb01360.x
Kruger A (1996) Chronic psychiatric patients' use of caffeine: pharmacological effects and mechanisms. *Psychol Reports* **78**: 915–923. doi: 10.2466/pr0.1996.78.3.915
Lachman LJ, Ravin L (1959). Complexation of benzocaine, procaine, and teracine with caffeine *J Am Pharm Ass* **45**: 120–123.
Larsen RW, Jasuja R, Hetzler RK, Muraoka PT, Andrada VG, Jameson M (1996) Spectroscopic and molecular modeling studies of caffeine complexes with DNA intercalators. *Biophys J* **70**: 43–452. doi: 10.1016/S0006-3495(96)79587-5
Lelo A, Birkett DJ, Robson RA, Miners JO (1986) Comparative pharmokinectics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol* **22***:* 177–182
Li Y, Feng Y, Zhu S, Luo C, Ma J, Zhong F (2012) The effect of alkalization on the bioactive and flavor related components in commercial cocoa powder. *J Food Comp Anal* **25**: 17–23
Leonard B (2000) Stress, depression and the activation of the immune system. *World J Biol Psych* **1**: 17–25.
Lindsay J, Laurin D, Verreault R, Hebert R, Helliwell B, Hill GB, McDowell I (2002) Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. *Am J Epidemiol* **156**: 445–453.
Mandal S, Mandal MD, Pal NK (2004) Evaluation of combination effect of ciprofloxacin and cefazolin against *Salmonella enteric* serovar *typhi* isolates by *in vitro* methods. *Calicut Med J* **2**: e2.
Matha A, Adbels OH (1982) Complex formation between antihistaminic drugs and caffeine. *J Am Pharm Ass* **9**: 208
Minamisawa M, Yoshida S, Takai N (2004) Determination of biologically active substances in roasted coffees using a diode-array HPLC system. *Anal Sci* **20**: 325–328. doi: 10.2116/analsci.20.325
Misumi M, Tanaka N (1980) Mechanism of inhibition of translocation by kanamycin and viomycin: a comparative study with fusidic acid. *Biochem Biophys Res Commun,* 92: 647–654
Mitchell DC, Knight CA, Hockenberry J, Teplansky R, Hartman TJ (2014) Beverage caffeine intakes in the US. *Food Chem Toxicol* **63**: 136–142. doi: 10.1016/j.fct.2013.10.042
Mohammed MJ, Al-Bayati FA (2009) Isolation, identification and purification of caffeine from *Coffea arabica* L. and *Camellia sinensis* L.: A combination antibacterial study. *Int J Green Pharm* **3**: 52–57. doi: 10.4103/0973-8258.49375
Mohanpuria P, Kumar V, Yadav SK (2010) Tea caffeine: metabolism, functions and reduction strategies. *J Food Sci Biotech* **19**: 275–287. doi: 10/1007/s10068-010-0041-y
Najafi NM, Hamid AS, Afshin RK (2003) Determination of caffeine in black tea leaves by fourier transform infrared spectrometry using multiple linear regression. *Microchem J* **75**: 151–158
Namboodiripad PA, Kori S (2008) Can coffee prevent caries? *J Conserv Dentistry* **12**: 17–21. doi: 10.4103/0972-0707.53336
Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Rev* **17**: 139–170. doi: 10.1016/0165-0173(92)90012-B
Nehlig A, Boyet S (2000) Dose-response study of caffeine effect of cerebral functional activity with a specific focus on dependence. *Brain Res* **858**: 71–77. doi: 10.1016/S0006-8993(99)02480-4
Olajuyigbe OO, Afolayan AJ (2012) *In vitro* antibacterial and time kill assessment of crude methanolic stem bark extract of *Acacia mearnsi* De Wild against bacteria in shigellosis. *Mol* **17**: 2103–2118. doi: 10.3390/molecules17022103
Osman F, McCready S (1998) Differential effects of caffeine on DNA damage and replication cell cycle checkpoints in the fission yeast *Schizosaccharomyces pombe*. *Mol Gen Genet* **260**: 319–334
Poehlsgaard J, Douthwaite S (2005) The bacterial ribosome as a target for antibiotics. *Nat Rev Microbiol* **3**: 870–881

Petersen PJ, Labthavikul P, Jones CH, Bradford PA (2006) *In vitro* antibacterial activities of tigecycline in combination with other antimicrobial agents determined by chequerboard and time-kill kinetic analysis. *J Antimicrob Chemother* **57**: 573–576. doi: 10.1093/jac/dki477

Pons FW, Muller P (1990) Induction of frameshift mutations by caffeine in *Escherichia coli* K12. *Mutag* **5**: 173–178. doi: 10.1093/mutage/5.2.173

Prescription drugs/caffeine interactions (2016) http://www.caffeineinformer.com/caffeine-drug-interactions. Accessed 01-05-2016

Pruthviraj P, Suchita B, Shital K, Shilpa K (2011) Evaluation of antibacterial activity of caffeine. *Int J Res in Ayuveda Pharm* **2**: 1354–1357

Raber J (1998) Detrimental effects of chronic hypothalamic-pituitary-adrenal axis activation. from obesity to memory deficits. *Mol Neurobiol* **18**: 1–22. doi:10.1007/BF02741457

Ramanaviciene A, Zukiene V, Acaite J, Ramanavicius A (2002) Influence of caffeine on lysozyme activity in the blood serum of mice. *Acta Med Lituanica* **4**: 241–244

Ramanaviciene A, Mostovojus V, Bachmatova I, Ramanavicius A (2003a) Anti-bacterial effect of caffeine on *Escherichia coli* and *Pseudomonas fluorescens*. *Acta Med Lituanica* **10**: 185–188

Ramanaviciene A, Acaite J, Dringeliene A, Markevicius A, Ramanavicius A (2003b) Effect of caffeine on mice immunocompetent cells. *Acta Med Lituanica* **2**: 86–89

Ribeiro JA, Sebastiao AM (2010) Caffeine and adenosine. *J Alzheimers Dis* **20** (Suppl 1): S3–S15. doi: 10.3233/JAD-2010-1379

Rodrigues CI, Marta L, Maia R, Miranda M, Ribeirinho M, Máguas C (2007) Application of solid-phase extraction to brewed coffee caffeine and organic acid determination by UV/HPLC. *J Food Comp Anal* **20**: 440–448. doi: 10.1016/j.jfca.2006.08.005

Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, Tung KH, Tanner CM, Masaki KH, Blanchette PL, Curb JD, Popper JS, White LR (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA* **283**: 2674–2679. doi: 10.1001/jama.283.20.2674

Sacks LE, Thompson PA (1977) Increased spore yields of *Clostridium perfringens* in the presence of methylxanthines. *Appl Environ Microbiol* **34**: 189–93.

Sakurai H, Mitsuhashi N, Tamaki Y, Akimoto T, Murata O, Kitamoto Y, Maebayashi K, Ishikawa H, Hayakawa K, Niibe H (1999) Interaction between low dose-rate irradiation, mild hyperthermia and low-dose caffeine in a human lung cancer cell line. *Int J Radiat Biol* **759**: 739–745. doi: 10.1080/095530099140087

Sandlie I, Lossius I, Sjâstad K, Kleppe K (1983) Mechanism of caffeine induced inhibition of DNA synthesis in *Escherichia coli*. *FEBS Lett* **151**: 237–242

Sawynok J (1995) Pharmacological rationale for the clinical use of caffeine. *Drugs* **49**: 37–50

Selby CP, Sancar A (1990) Molecular mechanisms of DNA repair inhibition by caffeine. *Proc Natl Acad Sci USA* **87**: 3522–3525

Suárez-Quiroz ML, González-Rios O, Barel M, Guyot B, Schorr-Galindo S, Guiraud JP (2004) Effect of chemical and environmental factors on *Aspergillus ochraceus* growth and toxigenesis in green coffee. *Food Microbiol* **21**: 629–634

Tornaletti S, Russo P, Parodi S, Pedrini AM (1989) Studies on DNA binding of caffeine and derivatives: evidence of intercalation by DNA unwinding experiments. *Biochim Biophys Acta* **1007**: 112–115

Traganos F, Kaminska-Eddy B, Darzynkiewicz Z (1991) Caffeine reverses the cytotoxic and cell kinetic effects of Novantrone (mitoxantrone). *Cell Prolif* **24**: 305–319. doi: 10.1111/j.1365-2184.1991.tb01159.x

Veselkov DA, Kodintsev VV, Pakomor VI, Djimant LN, Davies DB, Veselkov AN (2002) 1H-NMR analysis of heteroassociation of caffeine with antibiotic actinomycin D in aqueous solutions. *Biophys* **45**: 193–202

Vinod DR (2004) *Pharmacognosy and Phytochemistry*. Carrier Publication, 1st edn, vol II, pp 311

Wilmot M (2006) *In vitro* inhibition of phytopathogenic fungi by caffeine, tea catechins theanine, polyphenon g, black-, green- and rooibos tea extracts. Pretoria, South Africa: Pretoria University, MSc thesis

Yen WJ, Wang BS, Chang LW, Duh PD (2005) Antioxidant properties of roasted coffee residues. *J Agric Food Chem* **53**: 2658–2663. Doi: 10.1021/jf0402429

Youn JH, Gulve EA, Holloszy JO (1991) Calcium stimulates glucose transport in skeletal muscles by a partway independent of contraction. *Am J Physiol* **260**: C555–C561.