Antimicrobial and Antihaemolytic Activities of Crude Extracts of Some Commonly Used Tea and Coffee in Nigeria

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Journal of Tea Science Research, 2017, Vol.7, No.6 doi: 10.5376/jtsr.2017.07.0006
Received: 16 Mar., 2017
Accepted: 06 Apr., 2017
Published: 22 May, 2017

Abstract Current indiscriminate abuse of existing antibiotics in clinical and veterinary treatments lead to an upsurge in antimicrobial resistant strains of microorganisms and aggressive search for alternatives which are readily available, less expensive with little or no side effect. Tea and Coffee are beverages consumed daily in every household in Nigeria. This study examines the antimicrobial and anti-haemolytic properties of commonly available Tea and Coffee in Nigerian market. The antimicrobial potencies of the extracts were assessed through disc diffusion method on pathogens of both man and animal origin while the anti-haemolytic assay was carried out through colorimetric method. The extracts were slightly acidic at full strength and no antifungal property was observed. Broad spectrum and bactricidal effects were observed against Staphylococcus aureus, Salmonella pullorum, Shigella dysenteriae and Streptococcus pneumonia. These activities were concentration dependent. Very poor activity was observed against Escherichia coli. Bactericidal rate of coffee was at 6hrs but ranged between 18 and 24 hrs for tea extracts. Tea extracts greatly inhibited the haemolytic potential of alpha toxins while coffee performed poorly. Tea and Coffee could thus serve as supportive treatment for some bacterial infections without fear of side effects, since they are naturally taken as daily beverages.

Keywords Tea; Coffee; Antimicrobial; Anti-haemolytic; Toxins; Nigeria

Introduction
The need for new antimicrobial agents is on the increase and closely associated with the problems of emergence of strains that are resistant to most present day antibiotics. This resistance may be caused by loss of cell wall permeability to drugs or conversion of an active drug to an inactive derivative by enzymes produced by the resistant strains (Franklin and Snow, 1975; Reiner, 1984). Another contributing factor is the use, misuse and overuse of antimicrobial agents on a large scale which lead to development of resistant strains of microorganisms. However, nature has provided us with numerous substances that can enhance healing and also allow us to achieve optimal health without the extensive lists of side effects and nightmare problems that come with synthetic drugs. Tea and Coffee are good examples of these natural resources which people drink and inadvertently reaping the associated health benefits (Arora et al., 2009). There is an increased interest on the properties of some plant stimulant beverages, particularly, chocolate, coffee and tea. Properties like anti-adherence and anticariogenic efficacy in dental caries care have been investigated (Ferrazzano et al., 2009). The usefulness of tea and coffee has also been reported to involve the prevention of biofilm formation, a condition that precedes dental caries. This ability is related to the presence of compounds like caffeine, theophylline, fluoride, tannic acid and oxidative enzymes (Puja et al., 2011; Sura, 2016). Caffeine, which is natural alkaloid or xanthine alkaloid, commonly found in coffee beans, tea leaves, cocoa beans and cola nuts have been reported to be the active antimicrobial constituent (Nawrot et al., 2003; Nonthakaew et al., 2015). However, the amount of caffeine varies per plant products and are equally affected by extraction method employed (Guo et al., 2011; Salinas-Vargas et al., 2014). Caffeine evolved as a chemical produced by plants to kill microbes and insect parasites. Therefore, caffeine has powerful antimicrobial powers that are probably responsible for the ability of coffee and tea to staunch the MRSA infections. Regular consumption of Tea and Coffee have been reported by Matheson et al. (2011) to be associated with a likelihood of lower MRSA nasal carriage and thereby recommending it as a new, safe, inexpensive and easily accessible method to decrease MRSA nasal carriage. Tea contains substances with antimicrobial potentials
such as caffeine, theobromine, xanthine and tannic acid (Elvin-Lewis et al., 1980; Otake et al., 1991). Toda et al. (1989) reported the antimicrobial effect of coffee on Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Vibrio cholera, Vibrio parahaemolyticus and Yersinia enterolitica and attributed the bactericidal effect to the presence of tannic acid. Sharma et al. (2014) evaluated the antimicrobial and anti-adherence effect of coffee on Streptococcus mutans to glass surface and recorded efficacy even after 4 hours of coffee intake. Antimicrobial properties of Tea and Coffee when applied topically, either in vitro or in vivo has been demonstrated (Fuji et al., 2003; Yamada et al., 2003) however, little has been done on the antimicrobial activity of these beverages in Nigeria. This study aimed at assessing the antimicrobial effect of tea and coffee extracts against the common pathogenic bacteria of human and animal origin and the anti-haemolysin property of the extracts on exo-toxins produced by some selected pathogenic bacteria thereby proposing the possibility of incorporating tea and coffee to treatment of infectious bacteria diseases.

1 Materials and Methods
1.1 Sourcing of tea and coffee
All Tea and Coffee samples used in the study were procured from standard Super markets in Nigeria. Tole tea was donated by a friend from Cameroon.

1.2 Extract preparation
(a) Aqueous extraction:
Cold water: Twenty milligrams (20 mg) of Tea leaves and Coffee powder were suspended in 100 mls phosphate-buffered saline (PBS) and held at room temperature (35°C) for 3 hours. The mixture was then centrifuged at 10,000 rpm for 15 minutes according to the method of Toda et al. (1989).

Hot water: The same concentration of the suspension in above was held at 70°C in a water bath for 2 hrs and then centrifuged at 10,000 rpm for 15 minutes.

(b) Other Solvents:
Tea leaves and Coffee powder were suspended in absolute ethanol and Petroleum ether at 25% concentrations. The suspensions were held overnight at room temperature and then centrifuged at 10,000 rpm for 15 minutes.

1.3 pH Estimation of extracts
The aqueous solutions (extracts) obtained from above were tested for their pH values using pH meter.

1.4 Sources of microbial isolates used in the study
Candida albicans, Candida tropicalis and all bacteria isolates were obtained from (i) the Bacteriology Laboratory of Federal School of Medical Laboratory Science (ii) Diagnostic Division of NVRI Vom, Nigeria. All isolates were sub-cultured for purity and viability before use.

1.5 Assay of antimicrobial activity
Agar diffusion method was used. Bacteria suspension that matches 0.5 MacFarland turbidity standard was seeded on freshly prepared Mueller-Hinton agar plates. Wells (6 mm in diameter) were punched in the seeded medium using sterile stainless cork borer and were filled with 0.1 ml of the Tea and Coffee extracts using calibrated 1ml pipette. Control wells were filled with the same quantity of appropriate solvent. The plates were left for pre-incubation diffusion at room temperature for 30 minutes. The plates were then incubated aerobically at 37°C for bacteria and 28°C for the fungal isolates for 18 hrs and 72 hrs respectively. The diameters of resulting zones of inhibition were measured in millimetres.

1.6 Determination of bactericidal and bacteriostatic activities of the extracts
A 10 Log dilution of Tea and Coffee extracts were prepared in test tubes with sterile nutrient broth as diluent. 0.1 ml of the saline suspension of the organisms was aseptically dispensed into the tubes containing the varying extract concentration. The extract-organism mixtures were subcultured on freshly prepared Blood agar and Nutrient agar plates which were incubated and examined for growth. The least extract concentrations with no growth were taken as the MBC while concentrations with the least growth were the extracts MIC.
1.7 Determination of rate of bactericidal action of extracts on test organisms

Bacteria suspensions were diluted 1:200 with double strength nutrient broth. Equal volume (1 ml) of the dilution and extracts of Tea or Coffee were mixed and incubated at 37°C and at intervals (0 hr, 3 hrs, 6 hrs, 12 hrs, 18 hrs and 24 hrs), 0.1 ml of the mixture was spread on to two separate nutrient agar plates which were incubated for 18 hrs at 37°C. At the end of incubation period, the plates were checked for growth. The test was repeated for each extract-organism combinations.

1.8 Combined effect of tole and coffee extracts

Strips of filter paper were cut out (1x5 cm) and sterilized in hot air oven at 161°C for 1 hr. These were carefully and aseptically immersed in the appropriate extract and got excess extract drained off on another sterile filter paper. The filter papers soaked with extracts were placed at right angle to one another on a previously seeded Mueller-Hinton agar. The plates were incubated at 37°C for 18 hrs after allowing a room temperature diffusion of 2 hours.

1.9 Toxin extraction

β-haemolytic strains of Streptococcus pyogenes and Staphylococcus aureus were cultured in peptone water and incubated for 20 hrs at 37°C with intermittent shaking as described by Honda et al. (1976). The cultures were then filtered using Swiney membrane filter (0.45 nm pore diameter) to remove the bacteria. The filtrates which now contain the toxins were used for the haemolysin test.

1.10 Assay of haemolytic activity

Haemolytic activity was assayed using washed rabbit erythrocytes suspended in normal saline. Adjust the absorbance of the Spectrophotometer at 700 nm to 0.3. A volume (0.1 ml) of the Tea or Coffee extracts was mixed with 3 mls of the above 10% erythrocyte suspension. Toxin solution (0.1 ml) was added simultaneously. The change in absorbance (taken at intervals of 0, 5, 10, 15, 20, 25 minutes) was recorded at room temperature with spectrophotometer at 700 nm.

2 Results

Preliminary results showed that there was no significant difference between the hot and the cold water extracts’ antimicrobial activities. However, the antimicrobial activities of both Ethanol and Petroleum ether extracts were poor. Hence, hot water extracts (which simulate the consumption condition of the samples) were used for all other analysis. Aqueous extracts’ pH of the samples ranged between 4.6 for Tea and 5.0 for Coffee while their colours ranged between light brown for Lipton to black for Coffee as shown in Table 1. High antibacterial activities against Staphylococcus aureus, Streptococcus pneumonia, Shigella dysenteriae and Salmonella pullorum were demonstrated by all the extracts with zone of inhibition ranging between 16 mm and 24 mm. This is shown in Table 2. No antifungal activity was noticed. Table 3 showed that S. aureus and St. pneumonia cells were totally killed at a coffee concentration of 30% but inhibited at 20%. It took 80% concentration of Tole tea extracts to kill Sal. pullorum and 50% to kill St. pneumonia cell. The rates of bactericidal action of the extracts are shown in Table 4. In 6 hrs, coffee extract was able to totally kill all the four isolates while it was between 12 to 24 hrs for the tea extracts. Figures 1a and 1b showed the rate of inhibition of haemolytic activity by the extracts. Varying rate of haemolysis was observed for the toxins examined. Rate of haemolysis for S. aureus toxin was reduced to 0.004 / min by Tole and Lipton extracts, 0.003 / min by Highland and 0.008 / min by the coffee extract as against the control with the rate of 0.01 / min.

Table 1 Colour and pH values of the aqueous extracts of tea and coffee samples

| Samples     | Extract colour | pH of cold extracts | pH of hot extracts |
|-------------|----------------|---------------------|-------------------|
| Tole        | Brown          | 4.7                 | 4.6               |
| Lipton      | Light brown    | 4.6                 | 4.9               |
| Highland    | Brown          | 4.8                 | 4.7               |
| Coffee      | Black          | 5.0                 | 5.0               |
Table 2 Antimicrobial activities of tea and coffee extracts on some selected microorganisms

| Microorganisms          | Tole Zones of inhibition (mm) | Lipton | Highland | Coffee |
|-------------------------|-------------------------------|--------|----------|--------|
| *Staphylococcus aureus* | 26.0                          | 18.0   | 16.0     | 24.0   |
| *Escherichia coli*      | 0.0                           | 0.0    | 0.0      | 0.0    |
| *Corynebacteria sp.*    | 12.0                          | 10.0   | 10.0     | 8.0    |
| *Aeromonas sp.*         | 0.0                           | 0.0    | 0.0      | 0.0    |
| *Klebsiella pneumonia*  | 0.0                           | 0.0    | 0.0      | 0.0    |
| *Proteus vulgaris*      | 7.0                           | 0.0    | 0.0      | 0.0    |
| *Proteus mirabilis*     | 0.0                           | 0.0    | 0.0      | 7.0    |
| *Pseudomonas aeruginosa*| 13.0                          | 14.0   | 11.0     | 16.0   |
| *Shigella dysenteriae*  | 24.0                          | 18.0   | 20.0     | 28.0   |
| *Klebsiella aerogenes*  | 0.0                           | 0.0    | 0.0      | 0.0    |
| *Enterobacter sp.*      | 12.0                          | 0.0    | 10.0     | 14.0   |
| *Streptococcus pneumonia* | 16.0                        | 17.0   | 16.0     | 20.0   |
| *Salmonella pullorum*   | 18.0                          | 16.0   | 18.0     | 16.0   |
| *Pasteurella maltocida* | 10.0                          | 8.0    | 8.0      | 0.0    |
| *Candida albicans*      | 0.0                           | 0.0    | 0.0      | 0.0    |
| *Candida tropicalis*    | 0.0                           | 0.0    | 0.0      | 0.0    |

Table 3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts on some susceptible bacteria

| Organisms                  | MIC(MBC) of extracts in % | Tole | Highland | Lipton | Coffee |
|----------------------------|----------------------------|------|----------|--------|--------|
| *Staphylococcus aureus*    | 50(60)                     | 50(60)| 50(60)   | 20(30) |
| *Shigella dysenteriae*     | 60(70)                     | 60(70)| 60(70)   | 40(50) |
| *Streptococcus pneumonia* | 40(50)                     | 40(50)| 50(60)   | 20(30) |
| *Salmonella pullorum*      | 70(80)                     | 60(70)| 70(80)   | 40(50) |

Table 4 Rate of bactericidal action of the extracts on selected organisms

| Organisms                  | Time in hrs | Tole | Highland | Lipton | Coffee |
|----------------------------|-------------|------|----------|--------|--------|
| *Staphylococcus aureus*    | 24          | 18   | 18       | 6      |
| *Shigella dysenteriae*     | 24          | 12   | 24       | 6      |
| *Streptococcus pneumonia*  | 18          | 18   | 24       | 6      |
| *Salmonella pullorum*      | 12          | 18   | 18       | 6      |

Figure 1 Rate of inhibition of haemolytic activity by tea and coffee extracts; a. *Staphylococcus* aureus exotoxin; b. *Streptococcus* pyogenes exotoxin
3 Discussion

Extracts of tea and coffee samples tested in this study showed activity against both Gram positive and Gram negative organisms, thereby making their activity broad spectrum in nature. No activity was observed against the two Candida species (Candida albicans and Candida tropicalis) tested probably because the extracts are unable to penetrate the sterol in the fungal cell wall. However, activities against filamentous fungi like Aspergillus tamari, A. niger and A. fumigatus, where the caffeine content was said to inhibit spore germination have been reported by Kumar et al. (1995). This was in contrast with Gamba et al. (2013) who reported tea extracts activity against C. albicans. These different observations might be due to variation in sample source, batch of the tea examined and extraction procedure employed. Olosunde et al. (2012) reported intermediate sensitivity of ethanolic extracts of tea to four bacteria species and confirmed the antimicrobial activity of aqueous extracts of tea. The antimicrobial properties of the extracts could be as a result of tannin which was already known for antimicrobial potency. Sensitivity of S. aureus, Sh. dysenteriae and Sal. pullorum, and the resistance of E. coli to the extracts was similar to the work of Toda et al. (1989). However, Ariza et al. (2014) reported good antimicrobial activity of cocoa extracts against E. coli and therefore suggested regular intake of it for healthy living. Four (4) microbial isolates that had earlier shown sensitivity were used for the determination of bacteriostatic or bactericidal nature of the extracts to the isolates. These activities were concentration dependent. The rate of bactericidal action of the extracts was encouraging. Coffee extract was able to kill all tested isolates within 6hrs, however, tea extracts could only achieve this by 12th and 18th hrs for the same isolates. This knowledge will be a useful guide about dosage and frequency of administration should these extracts be considered for therapeutic purpose. Regular consumption of coffee reduces growth rate and adherence of microbe to tooth surface and also have anti-demineralization effect on teeth thus, opening hope for future application (Signoretto et al., 2010; Antonio et al., 2011) Extracts were examined for the inhibition of haemolytic activities of S. aureus and St. pyogenes exo-toxins. These cytotoxic substances are believed to be the major cause of tissue damages during staphylococcal infections (Bernheizer, 1974). The cytolytic effect of the toxins is achieved through formation of transmembrane hollow or pore in the plasma membrane of susceptible cell through which intracellular materials leak out leading to cell death (Fussle et al., 1981; Cassidy et al., 1974). Therefore, the ability of tea extracts to inhibit haemolysis caused by the toxins can be related to its ability to prevent the formation of transmembrane pores through which intracellular materials leak out. Tole, Lipton and Highland tea extracts showed marked (though not complete) antihemolytic activities against the toxins. Coffee inhibited activity only slightly in agreement with the work of Sachie et al. (1989). The substance responsible for the anti-haemolysin activity of tea may be catechins, because studies have shown that catechins purified from green tea also inhibited the haemolytic activities of Staphylococcus α-toxin and Vibrio parahaemolyticus’ toxin (Sachie et al., 1989). Other studies suggested that flavonoids could be incorporated in erythrocyte membranes and improve their stability or that the exacerbation of the van der Waals contacts inside the lipid bilayer to flavonoids could be a source of membrane stabilization (de Freitas et al., 2008; Mhamed et al., 2015). This shows that extracts of tea can be used in treating some haemolytic diseases. Tole and coffee gave a synergistic effect in vitro but their use together in vivo needs additional studies.

4 Conclusions

Tea and coffee aqueous extracts demonstrated antibacterial activity against microorganisms of both human and animal, which means even animal diseases, could equally be treated with them. By their anti-haemolytic potency, tea extracts could be used to reduce severity associated with food poisoning or other forms of haemolytic disease. Continuous and regular intake of tea and coffee is highly encouraged.

Acknowledgments

I appreciate the contributions of Late Pof. GO Nwobu, Mrs Sarah Olajubu and all the Technical staff of Microbiology Laboratory, AAU and Bacteriology Unit of NVRI Vom, Nigeria.

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