

## Research Article

### Composite Astringent Index and the Determination of Anti-infective Properties of Selected Folklore Therapies

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#### ABSTRACT

**Purpose:** An attempt was made to quantify comparative astringent powers among selected substances taking tannic acid as the reference standard. The measurements were to be based on composite astringent power of all contributing compounds present in extracts. Among the folklore antimicrobial measures, consumption of black tea and black coffee in diarrheal conditions are well established. Their effectiveness was to be determined through demonstration of reduction in bacterial colony counts. **Method:** Astringent power of selected materials were determined using standard egg albumin solution by titrating with different astringent preparations. End points were determined when protein precipitation was completed using UV absorption measurements and biuret test. To evaluate anti-microbial activity of black tea and black coffee infusions, experiments on growth inhibition by a reduction of colony forming units were carried out. **Results:** The strongest astringent activity was found with *Terminalia chebula* (aralu) extract and the weakest with coriander extract. Black tea and black coffee infusions showed a high percentage of inhibitory activity against both *Escherichia coli* and *Salmonella typhi* in a range 70.83%±3.6 to 87.50%±0.00. **Conclusion:** Using astringent power of substances, the Composite Astringent Index was established. It was clear that consumption of black tea and black coffee infusions during diarrheal states can achieve significant anti-diarrheal effects. Materials with greater astringency showed greater anti-diarrheal effects. Materials with minimum end point values for the protein precipitation assay yielded the lowest astringent power values.

**Key words:** Composite Astringent Index; Protein precipitation; Black tea; Black coffee; Folklore medicine.



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## INTRODUCTION

Astringency is an important flavor sensation and is produced by a variety of oral chemical stimuli, including tannins and other polyphenols.(1)

The current research was carried out to investigate the astringency of selected astringent materials and the validity of some folklore anti-infective measures against bacteria. Astringent activity is a kind of a chemical reaction having specific activity to shrink or coagulate protein within the body tissues.(2) The word astringency is derived from the Latin *adstringere*, which means “to bind fast”.(2) Furthermore, in medicine, astringency causes contraction of mucus membranes, of any exposed tissues and are often used internally to reduce discharge of blood serum and mucus secretions.(3) Externally applied astringents lead to mild precipitation of skin proteins. Due to astringent effects of drying and hardening, the skin is protected.(2) Therefore, astringency produced by many plant materials have been used in traditional medicine for various conditions.

The perceived astringency caused by polyphenols has been extensively studied by previous researchers. Oral astringency caused by polyphenolic compounds such as tannins have been attributed to the formation of complexes with salivary proteins and mucopolysaccharides.(4) Most of the astringent materials contain mild to high levels of tannins as the prominent constituent. The astringent effect of tannin causes the dry and puckery feeling in the mouth following the consumption of plant materials containing high amounts of tannins present in products

such as red wine, strong tea or unripe fruits.(4) Some fruits and their parts including rhubarb, quince, bird cherry, and banana skins are found to be astringent. In addition, some common plants with astringent properties are acacia, witch hazel, bayberry and sage yarrow. Zinc oxide preparations, lacto calamine lotion, tincture of benzoin are also considered as astringent preparations.(4)

Accordingly, it was felt that a well-defined scale on the degree of astringency among astringent materials should be established. Therefore, the current research was designed to establish a ‘Composite Astringent Index’ for astringent materials with respect to tannic acid. Tannic acid was used as the reference standard, because tannins are the predominant chemical constituent present in the majority of astringent material.(5)

Folklore beliefs have been practiced throughout generations by transmission of knowledge orally. This knowledge has been based on the properties of medicinal plants which are the most ancient source of curing human illnesses. The recognition of their therapeutic action through vast amounts of empirical knowledge concerning the treatment of diseases is the fundamental basis of traditional medicine. During the last two decades, the use of traditional medicine has been expanded globally, gaining popularity. It has continued to be used not only for primary healthcare of the poor in developing countries but also in countries where modern medicine is predominant.(6) Furthermore, traditional medicine is fully integrated into the health systems in China, North and South Korea and Vietnam.(6) In India, 70% of the population use the Indian traditional

medicine.(7) Hence it is clear that today, there is a great deal of interest in traditional medicine around the globe mainly due to the realization of the harmful side effects of many allopathic (Western) medicines.

Anti-infective properties of many herbs have been documented in ancient manuscripts. Among them, few are investigated for their antimicrobial activities.(8) The folklore anti-infective measures are against the infectious agents which include bacteria, fungi, protozoa and viruses. The ancient people used to gargle the throat with concentrated solutions of common salt to treat throat and gum abscess, believing this treatment would induce rupture of abscesses.(9) Moreover, in order to kill the pathogen and to accelerate the process of curing, a mixture of scraped coconut or sugar is placed on old wounds. Both the made tea and black coffee infusions are used as anti-diarrheal agents due to their antimicrobial activity. In folklore medicine, roasted spice (Badapu thunapaha) is also used as a treatment for diarrhea.

Another traditional anti-infective measure is to draw the sap, from the end of 10-15 inflorescences of love grass (Thuththiri) and place it on a sty that appears on an eye lid and expose to morning sunlight, believing that it may kill the organisms in the sty. In addition, people in the villages also used coconut stud paste (Gobalu) and a paste made from young leaves of *Coffea arabica* as a treatment for wound infections and abscesses. In this case, they rubbed the tender stalk end of coconut stud to make a paste and it was applied on the infection site. Asafetida (Perumkayam) is used as an anti-infective measure to prevent air borne infections. By placing it under the

pillow of an infected person, people believe that spreading of pathogens through air would minimize.(9) Apart from that, they used it as an atmospheric disinfectant. Similarly, the leaves of *Azadirachta indica* (Kohomba) also have the atmospheric disinfectant property and in ancient practice people used to hang the leaves at the front door of the infected house, especially in houses infected with diseases thought to be of divine origin.(10) The *Ocimum canum* (Maduruthala) leaf, lemon grass leaf or fumes of dried cashew nut peel were used as mosquito repellents especially in households infected with diseases believed to be due to divine origin or curse.(11)

The two main objectives of this study were to experimentally determine the effectiveness of astringent plant materials such as black tea and black coffee as anti-infective agents, and to determine the comparative astringent powers of selected materials and establish a 'Composite Astringent Index' for such substances.

## METHODS

Analytical grade aluminum chloride, zinc chloride (Sigma Chemical Co) and tannic acid (ACS reagent brand Sigma-Aldrich) were used in the experiments. Albumin stock solution and Biuret reagent (Laboratory Grade of brand SCIED) were prepared according to the laboratory standards.(12) Authenticated dried plant materials of Aralu (*Terminalia chebula*), Bulu (*Terminalia Bellirica*) and Nelli (*Emblica officinalis*) were obtained from the Link Natural (Pvt) Ltd, Kapugoda, Sri Lanka. Brocken Orange Pekoe (BOP) 2 grade black tea (*Camellia sinensis*) was obtained from Morawakkolare Tea Manufacturing

Cooperation, Deniyaya, Sri Lanka. Green colored coffee beans (*Coffea arabica*) were obtained from the local market which were later roasted and crushed in to a powder.

Equipment used included electronic balance (CAS-MWP-600H Japan) and Ultra violet spectrometer (Cary 60 UV-Vis, Agilent Technologies, Singapore). Microbiological experiments for *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 25923) were conducted at the Department of Quality Control, State Pharmaceutical Manufacturing Corporation, Kandawala Estate, Ratmalana.

### Experiments on Composite Astringent Index

**Preparation of tannic acid reference solution:** Two parts (2g) of tannic acid were dissolved in 150 parts (150 ml) of warm purified water.

**Preparation of extracts of astringent test material:** Two parts (2 g) of selected plant material, (black tea, black coffee, nelli, coriander, bulu, aralu) were taken and each were boiled in 150 parts (150 ml) of purified water separately and were held for 15 minutes. Lacto calamine lotion, aluminum chloride solution, and zinc chloride solutions were also prepared in the same proportions but without boiling to serve as mineral origin astringent material in the study.

### Preparation of standard egg albumin solution 10% w/v.

Colorless egg albumin was collected without mixing with egg yolk. Five parts (5 g) of colorless fluid fraction leaving out the gel like strands was weighed in to a 100 ml beaker. Then 50 parts (50 ml) of purified water was

added and was gently shaken to homogenize. This served as the albumin standard stock solution freshly prepared each time for the test.

**Protein Precipitation Assay (PPA) for selected astringent materials:** The protein precipitation assay was done by slight modification of the method described in the reference.(13) Prior to the modification, a pilot study was carried out to determine the possible endpoint of the protein precipitation titration reaction. One ml aliquot of each of the prepared astringent solutions were added separately at a time in to each 50 ml of the 10% (w/v) test solution of egg albumin. At each 1 ml addition, the reaction mixture was gently shaken and left for 10 minutes. The pH of the reaction mixture at each titration step was determined. Then 2 ml of the supernatant of that mixture was withdrawn. One ml was for the Biuret test and other one ml was to determine UV-Vis absorption at  $\lambda$  maxima of 280 nm. This procedure was continued until the approximate end point was reached after which progressively smaller volumes were added. The volume consumed at the end point was recorded. Then corresponding UV absorption values verses the volume consumed and pH variation against the volume consumed graphs were sketched. By curve fitting, accurate end point values were obtained. The tests were repeated in triplicate for all the astringent test samples. In order to determine end points of the standard tannic acid reference solution, the same test procedure was repeated.

**Determination of astringent power by the Protein Precipitation Assay (PPA):** The astringent power was determined by an in-

house method developed by our research group. The astringent power is defined as follows in this study.

“It is the value obtained by dividing the volume of an infusion representing total astringent power made with two parts by weight (g) of the sample in 150 parts by volume (ml) of purified water brought to

boiling and then held for 15 minutes, that completely precipitate 50 ml of a 10% (w/v) solution of egg albumin, with the volume similarly obtained with a solution of two parts by weight (g) of the tannic acid in 150 parts by volume (ml) of boiling purified water held for 15 minutes”.

$$\text{Astringent Power} = \frac{\text{Consumed volume of the test sample against standard albumin solution}}{\text{Consumed volume of standard tannic acid solution against standard albumin solution}}$$

Individual astringent powers of astringent materials were calculated by using the end point consumption values of the PPA. Using this formula astringent power of the materials were determined with respect to the PPA end point of tannic acid standard. Composite Astringent Index was established using these values.

**Experiments on folklore anti-infective preparations:** The preparations were made using domestic quantities popularly employed for the purpose.

**Preparation of black tea infusion:** Sterile tea infusion was prepared according to Ceylon Tea Board guidelines (14) with minor modifications. In folklore practice, the tea extract should be of a higher concentration than regular use for anti-diarrheal effects. Therefore, it was taken as 1.5 times the concentration of usual use. Three gram (3g) of black tea leaf was boiled in 150 ml water to produce black tea extract. The container was covered and left to stand for about 15 minutes. The infusion was then transferred into a sterile beaker.

**Preparation of black coffee infusion:** Green color coffee beans were collected (*Coffea arabica*) from the local market. Then beans were roasted until it became brown. Then beans were grounded to obtain fine coffee powder. According to the National Coffee Association (USA) guidelines (15), two tablespoons of ground coffee was added in to 150 ml of boiling water and was left to stand for about 15 minutes to prepare the coffee infusion. Then the prepared coffee infusion was transferred in to a sterile beaker.

**Preparation of Tryptic Soy Agar (TSA) medium:** Tryptic Soy Agar (TSA) powder (28 g) was suspended in 1 liter of distilled water. Thereafter, the resultant mixture was dissolved by applying heat. Adjusted the pH to  $7.6 \pm 2$  and sterilized by autoclaving at  $121^\circ\text{C}$  at 15 psi pressure for 15 minutes. After TSA had been autoclaved, it was allowed to cool but not solidify. The TSA was transferred into each plate and the plates were allowed to stand on the sterile surface until the agar had solidified. The lid of each Petri dish was replaced and the plates were stored in a refrigerator at  $2-8^\circ\text{C}$ .

### Preparation of standard microbial suspensions:

First generation of sub-cultured *E.coli* standard strain colonies containing agar plate was taken. Three colonies of sub-cultured *E.coli* were transferred aseptically by taking them from an inoculation loop, they were introduced to the pre prepared TSA slant media by streaking and was incubated for 24 hours at 32<sup>0</sup>C. Thereafter, all the colonies present on the surface of TSA slants were scraped off and was introduced in to 20 ml of normal saline solution (0.9 w/v NaCl). Finally the resultant *E.coli* suspension was homogenized by vortex. After that 1 ml of resultant suspension was added to a sterile test tube and was diluted up 10 ml by using sterile normal saline solution. This procedure was repeated till the initial concentration of the suspension became 10<sup>-9</sup> per ml dilution. This served as the dilution series for the *E.coli* suspension. One (1 ml) volume of each diluted *E.coli* suspension was transferred to a sterilized petri dish, molten TSA was added and was rotated clockwise and anti-clockwise for at least two times. The plates were incubated at 32<sup>0</sup>C for 24 hours. After incubation for 24 hours, TSA plates were observed for any microbial growth. Number of countable colonies per dilution was considered as 25-100 cfu/ml for the acceptable range. The standard microbial suspension for *Salmonella typhi* was also prepared as the same method as describe above.

### Methodology of determination of indicative anti-diarrheal activity of black tea and black coffee:

According to the scheme in Table 1, the respective test materials were added from T1-T8 test tubes. Then all test tubes were incubated at 37<sup>0</sup>C and were

allowed to stand for three hours for microbial inhibition activity. This represents the gastro intestinal dwelling time after consumption of tea by a person. One ml volume of each resultant test solution (T1-T8) were transferred to a sterilized petri dish and then molten TSA was added.

**Table 1: The test scheme for indicative anti-diarrheal activity**

Test material	T1	T2	T3	T4	T5	T6	T7	T8
Normal saline solution*	+	+	-	-	-	-	-	-
Black tea infusion**	-	-	-	-	+	+	-	-
Black coffee infusion***	-	-	-	-	-	-	+	+
Tannic acid****	-	-	+	+	-	-	-	-
<i>E.coli</i> standard solution #	+	-	+	-	+	-	+	-
<i>Salmonella</i> standard solution##	-	+	-	+	-	+	-	+
	Negative control		Positive control		Test samples			

\*Volume of normal saline solution added in to each test tube (30 ml).\*\*Black tea infusion volume (30 ml).\*\*\*Black coffee Infusion volume (30 ml). \*\*\*\*Equal amount of tannic acid which present in 30 ml of black tea extract was added according to the tannic acid strength. (\*Tannic acid amount was determined by calibration curve). #*E.coli* stranded microbial suspension volume (1 ml), ##*Salmonella* stranded microbial suspension volume (1 ml).

The plates were incubated at 32<sup>0</sup>C for 24 hours. After incubation for 24 hours, TSA plates were observed for any microbial growth. Two additional agar plates were incubated without introducing any reagent as

a neutral control to investigate interference by TSA medium. After the determination of the colony forming unit on the respective TSA plates (T1-T8) the microbial counts of test samples were compared with positive control.

Finally, the Percentage Growth Inhibitory Activity (PGIA) was determined. PGIA calculation was done according to the equation formulated by our research group which is given below.

$$\text{Percentage growth inhibitory activity} = \frac{(\text{cfu in negative control} - \text{cfu in test sample})100\%}{\text{cfu in negative control}}$$

## RESULTS

### Determination of astringent power by the Protein Precipitation Assay (PPA)

According to the results obtained in PPA (Table 2) the maximum end point consumption volume observed for the materials of plant origin was 23.0±0.5 ml for coriander and the minimum was 3.4±0.8 ml for aralu. Among other material, the maximum end point value of 8.4±0.3 ml was for lacto calamine lotion and the minimum of 4.2±0.5 ml was for aluminum chloride as mineral astringent materials (Table 2).

According to this table, it was observed that the end point volume from absorbance data and pH deflation data had a significant correlation. The lowest Composite Astringent Power is with aralu (0.76) and the highest is with coriander (5.11). Astringent power values are unit less, smaller the value stronger is the activity. Astringent power values of test samples were listed together forming the Composite Astringent Index (Table 2).

**Table 2: End points in Protein Precipitation Assay, pH deflations and astringent powers**

Materials	End point (ml)	pH deflation point (ml)	Composite Astringent Index (Astringent power = Test sample/tannic acid)
Coriander	23.0±0.5	22.0±0.3	5.11
Bulu	11.4±0.5	11.5±0.7	2.53
Black Tea	10.5±0.3	11.0±0.8	2.33
Lacto-calamine lotion	8.4±0.3	9.0±0.5	1.87
Zinc chloride	6.0±0.3	5.5±0.4	1.33
Tannic acid	4.5±0.4	-	1.00
Nelli	4.4±0.5	4.7±0.4	0.97
Black coffee	4.3±0.7	4.4±0.3	0.95
Aluminum chloride	4.2 ±0.5	4.8±0.5	0.95
Aralu	3.4±0.8	3.0±0.8	0.76

### Determination of indicative anti-diarrheal activity of black tea and black coffee

Percent Growth Inhibitory Activity (PGIA) of black tea and black coffee infusions against *E. coli* and *S. typhi* are given in Table 3. The PGIA against *E.coli* was  $70.83\pm 4.16\%$  for black tea and  $87.50\pm 4.17\%$  for black coffee. The PGIA against *S. typhi* was  $60.71\pm 3.57\%$

for black tea and  $82.50\pm 3.51\%$  for black coffee. It was observed that black coffee has maximum percentage inhibitory activity for both *E.coli* and *Salmonella*. It was noted that the black coffee activity is similar to that of standard tannic acid solution which is the positive control.

**TABLE 3: Colony forming units and Percentage Growth Inhibitory Activity (PGIA)**

Test	Count (cfu /ml)	Percentage activity
<b>T1 (<i>E.coli</i> + Normal saline)</b> (Negative control)	24±3	0.0%
<b>T2 (<i>Salmonella</i> + Normal saline)</b> (Negative control)	28±2	0.0%
<b>T3 (<i>E.coli</i> + Tannic acid)</b> (Positive control)	4±1	83.33±4.17%
<b>T4 (<i>Salmonella</i> + Tannic acid)</b> (Positive control)	5±2	82.14±7.14%
<b>T5 (<i>E.coli</i> + Black tea )</b>	7±1	70.83±4.16%
<b>T6 (<i>Salmonella</i> + Black tea)</b>	11±1	60.71±3.57%
<b>T7(<i>E Coli</i> + Black coffee)</b>	3±0	87.50±4.17%
<b>T8 (<i>Salmonella</i> + Black coffee)</b>	5±2	82.50±3.51%
<b>Neutral control</b>	0±0	-

### DISCUSSION

During the titration of individual astringent extracts in to egg albumin solution, the pH value of the egg albumin gradually decreased and became constant at a particular volume of titrant. The corresponding volume was labeled as the pH deflection point. However, in order to get a clear understanding about the relationship between pH and the astringency, a separate research study must be carried out since the acidity of the sample can interfere with the end point.

Also, this research was conducted to investigate the scientific basis of selected folklore measures which is utilized in diarrheal conditions. The aim of this work was to evaluate anti-infective activity using well-defined microbiological procedures. Accordingly, the validity of *in-vitro* anti-infective measures of Black Coffee Infusion (BCI) and Black Tea Infusion (BTI) in the treatment of diarrhea was tested. In order to represent the *in-vitro* anti-diarrheal activity, *Salmonella typhi* and *Escherichia coli*, were selected as the common causative organisms

for diarrhea in adults.(16) According to the test procedure,  $33 \times 10^8$  cfu/ml for *E. coli* Standard Microbial Suspension (ESMS) and  $37 \times 10^8$  cfu/ml for *S.typhi* Standard Microbial Suspension (SSMS) were used as the inoculums. In the determination of both Composite Astringent Power and the PGIA, Tannic Acid Standard Solution (TASS) was used as the reference solution and the positive control respectively. It is important to compare the astringent activities of the selected samples and PGIA of BTI and BCI against tannic acid since it is the popular astringent material of natural origin and closely relates to ethno-botanical folklore therapies. On the other hand, Table 2 shows that all three mineral samples too showed powerful astringent activities closer to and in the case of Aluminum chloride, stronger than the TASS. The use of zinc insulin complexes and aluminum-antigen complexes in vaccine technology are two well established instances of this interaction.

## CONCLUSION

By using the parameter astringent power (AP), astringency of a given material can be evaluated in a quantitative manner. It must be noted that higher astringent power values represent lower astringent activity according to our definition. Using the AP data of materials, we succeeded in developing the Composite Astringent Index for the different materials, which can be used to analyze the

comparative astringency of any substance. It was also suggested that the anti-bacterial effect of BTI and BCI is due to their astringency.(17) This is proved with the current findings also. This is justified with approximate comparable Percentage Growth Inhibitory Activity (PGIA) observed in TASS with those of BTI and BCI (Table 3). Therefore, maximum PGIA was observed in materials with minimum AP value. Furthermore, the effectiveness of the consumption of black coffee and black tea in diarrheal conditions is proved by this research study. The most important message is the availability of alternative antimicrobial measures to that of classical antibiotics. These include astringency, hypertonic salt or sugar, fermentation and media with extreme pH all of which are employed in folklore practices.

**Competing interests:** The authors declare that they have no competing interests to disclose.

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## REFERENCES

1. Gowel R, Oberholster A. and Francis, I.L A 'Mouth-feel Wheel': Terminology for Communicating the Mouthfeel Characteristics of Red Wine, Aust J Grape Wine Res. 2000;6:203-7.
2. Bagyalakshmi B. and Balamurugan A. (2017). Inhibitory Activity of fresh green tea and Black tea extracts (*Camellia*

- sinensis*) on Internal Pathogen Isolated from Diarrhea. International Research Journal of Pharmacy. 2017;8(10):93-8.
3. Sakhawat H, Nisarath N, Shadhan KM, Zia UM, Shafiqul IS Amran K et al. Phytochemical Analysis and Antioxidant, Thrombolytic, Astringent, Pro-Coagulant Properties Investigation of *Tagetes lucida*. Pharmacology Online. 2019;3:203-10.
  4. Lee C, Ismail B, Vickers Z. The Role of Salivary Proteins in the Mechanism of Astringency. Journal of Food Science. 2012;77(4). <https://doi.org/10.1111/j.1750-3841.2012.02644.x>
  5. Ashok PK, Upadhyaya K. Tannins are Astringents. Journal of Pharmacognosy and Phytochemistry, 2012;1(3):45-50.
  6. WHO. 'A vision for Primary health care in the 21st Century', World Health Organization. 2018. Available at: <https://www.who.int/docs/default-source/primary-health/vision.pdf>
  7. Pandey MM, Rastogi S, Rawat AKS. 'Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation', Evidence-based Complementary and Alternative Medicine, 2013. doi: 10.1155/2013/376327.
  8. Ghasemi PA, Malekpoor F, Enteshari S, Yousefi M, Momtaz H, Hamedi B. Antibacterial Activity of Some Folklore Medicinal Plants Used by Bakhtiari Tribal in Southwest Iran. International Journal of Biology. 2010;2(2):55-63 doi: 10.5539/ijb.v2n2p55
  9. Martin K. Salt Water Gargle-Martin Kevin. 2016. Available at: <https://patents.google.com/patent/US20160346322A1/en> [Accessed 1 November 2020].
  10. Alzohairy M. A. 'Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment'. Evidence Based Complementary and Alternative Medicine. 2016. <http://dx.doi.org/10.1155/2016/7382506>
  11. Ntonga PA, Baldovini N, Mouray E, Mambu L, Belong P. et al. 'Activity of *Ocimum basilicum*, *Ocimum canum*, and *Cymbopogon citratus* Essential Oils Against Plasmodium Falciparum and Mature-Stage Larvae of *Anopheles funestus*. 2014; 21:33. doi: 10.1051/parasite/2014033.
  12. Kit, T. P. et al. 'Technical bulletin', pp2-4.
  13. Hagerman AE. Radial Diffusion Method for Determining Tannin in Plant Extracts. Journal of Chemical Ecology. 1987;13(3):437-49.
  14. Ceylon tea. Sri Lanka Tea Board Standards /Guidelines for Tea. 2020 Available at: [http://www.pureceylontea.com/images/sltb\\_downloads\\_new/Sri\\_Lanka\\_Tea\\_Board\\_Standards\\_Guidelines\\_for\\_tea.pdf](http://www.pureceylontea.com/images/sltb_downloads_new/Sri_Lanka_Tea_Board_Standards_Guidelines_for_tea.pdf)
  15. National Coffee Association. How to Brew Coffee. Available at: <https://www.ncausa.org/About-Coffee/How-to-Brew-Coffee>.
  16. Mackay BJ, Denepitiya L, Iacono VJ, Krost SB, Pollock JJ. Growth-Inhibitory and Bactericidal Effects of Human Parotid Salivary Histidine-Rich Polypeptides on *Streptococcus* mutants. Infection and Immunity. 1984;44(3):695-701. doi: 10.1128/IAI.44.3.695-701.1984
  17. Gerald WD, Ming S, Craig McC. Action of Polyphenols of Green Tea Against Gastrointestinal Diseases. Current Opinion in Gastroenterology. 2006;22(2):165-170. doi: 10.1097/01.mog.0000208463.69266.8