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Research Article

Astringent activity of folklore plant materials and selected formulations in relation to their antimicrobial activity

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ABSTRACT

Purpose: The astringent activity of tannins is due to their ability to bind with proteins which in turn contributes to their antimicrobial properties. The study aimed at determining the correlation between the composite astringent power of folklore plant materials and selected herbal formulations in relation to their antimicrobial activity. Protein precipitating ability (Astringent Power) and Tannic Acid Astringent Equivalents of extracts of the materials were determined.

Method: A protein precipitation assay was designed to determine the Tannic Acid Astringent Equivalents of the samples. Antimicrobial activity was determined by agar disc diffusion assay with *Escherichia coli* and *Staphylococcus aureus.*

Results: Folklore materials and formulations showed strong negative correlation coefficients with Tannic Acid Astringent Equivalent values and antibacterial activities. Values observed for the folklore materials were, *S. aureus* (r = -0.937) and *E. coli* (r = -0.825) and for the formulations *S.aureus* (r = -0.9825) and *E.coli* (r = -0.8774). A new term 'very tender coconut' was introduced to describe *Gobalu* (Sinhala).

Conclusion: The folklore materials and marketed formulations have a strong correlation between composite astringent activities with their antimicrobial powers. High astringent activity corresponds with high antimicrobial activity justifying their ethnobotanical medicinal applications.

Key words: Folklore plant materials, Composite astringent power, Tannic Acid Astringent Equivalent (TAAEq), Tannic Acid Astringent Equivalent Index, Very tender coconut.

INTRODUCTION

"Astringency" is a tactile sensation. The American Society for Testing and Materials defines astringency as "the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of

exposure to substances such as alums or tannins". (1) The astringency of tannins found in plant extracts is responsible for the dry and puckery feeling in the mouth. Tannins are plant polyphenols, which have high molecular weights and contain sufficiently high hydroxyl groups. They form strong complexes, precipitating proteins.(2) Enzymes are vital functional proteins in all biological systems. Tannins can interfere with these enzymes, which contribute to their broad spectrum of antimicrobial properties in all biological systems.(3,4) Tannins have a strong protein coagulation action and their antibacterial effects have been recognized for a long time.(5)

In Sri Lanka, folklore medicines have been practiced for over 3000 years. Folklore medicines used in Sri Lanka are a combination of the indigenous medicine of the country and the Ayurveda system of India. (6) Folklore medicines play a pivotal role in the rural population of Sri Lanka by fulfilling 60%-70% of their primary healthcare needs.(7) In folklore medicine, traditional knowledge of natural herbs practiced throughout the generations, for thousands of years, to cure and treat illness is utilized. (8,9)

Plant materials are a huge chemical storehouse of undiscovered biodynamic compounds. (10-12) Scientists have great interest in biologically active compounds of natural sources which are used to treat infectious diseases. Alkaloids, tannins, phenolic compounds, flavonoids, and phenanthrenes are the most significant bioactive compounds in plants with *in vitro* antimicrobial activity. (13,14)

People have been using cosmetics and other formulations since ancient times. (15,16) Various types of formulations used during skin ailment to provide action such as skin protection, antiacne, sunscreen, antiaging, and anti-wrinkle are designed using several material, either natural or synthetic. (17) Many commonly marketed formulations people use in day-to-day life such as toothpastes, deodorants and creams possess antimicrobial activity. (18-20)

Bacteria living on the skin cause unwanted odor mainly in the axilla area and the feet with sweating. Volatile fatty acids like compounds that are metabolized by those bacteria are found in sweat, lead to the development of unwanted body odor. Reducing the quantity of sweat and inhibiting odor-producing bacteria are the most commonly used methods to deodorize. Aluminium Potassium Sulfate Dodecahydrate ($2KAl(SO4)_2.12H_2O$) containing deodorants, creams, and after shavings have an astringent effect on the skin, tightening the sweat glands which are further narrowed and also inhibit body odor-causing bacteria**.** (21)

Among the terms introduced in the study, the ‘Astringent Power’ of a given sample was simply assigned based on the endpoint volume of the sample consumed by a standard egg albumin solution in the protein precipitation assay, stronger items requiring smaller volumes. ‘The Composite Astringent Power’ of an extract or formulation refers to the sum total of astringent activities of all ingredients present, as against many modern studies on single ingredients. These two terms may coincide in many instances. Comparative astringent power is expressed in terms of ‘Tannic Acid Astringent Equivalent’ (TAAEq). It is defined as “The ratio of the consumed volume of a specified extract of the sample at the endpoint against egg albumin stock solution in relation to the consumed volume of the tannic acid standard solution against the egg albumin

stock solution under defined conditions. 'Astringent Index' is the orderly listing of TAAEq values. (22)

Tannic acid is considered as the reference substance since it is the predominant chemical compound that can be found on most astringent materials. According to our definition, if a particular sample has high astringency (protein precipitating ability) its astringent power in milliliters takes a low value. It requires a small volume to precipitate a standard amount of egg albumin solution. If a particular sample has low astringency, its astringent power value is high. It consumes a larger volume to precipitate the standard amount of egg albumin solution. (22)

Although traditional Sri Lankan society has been practicing the folklore plant medicines for thousands of years, the validity and the scientific basis behind the use of plant medicines remained unresolved. The aim of this study was to investigate the validity and the scientific basis behind the antimicrobial activity of selected folklore materials and to index their astringent activity using well defined scientific procedures including the newly introduced TAAEq. (22)

The study also included the astringent activity and antimicrobial activity of marketed formulations of toothpaste, Calamine powder, and herbal sachet for reconstitution before oral administration. Correlation between and TAAEq and the antimicrobial activities of selected plant materials and their formulations were also established.

MATERIALS AND METHODS

The folklore materials studied were Neem leaves (*Azadirachta indica), Perumkayam* (Asafetida powder), Roasted Spice Mixture (Containing the seeds of *Coriandrum sativum, Cumin cyminum, Foeniculum vulgare*), 'Very Tender Coconut' or *Gobalu* in Sinhala (*Cocos nucifera* Linn; Typical Tall Variety) (23) and Tender Coconut Water obtained from the tender shell coconut fruits of the Typical Tall Variety. Very tender coconut refer to the youngest coconuts, which emerged fresh from the inflorescence and grown to about 3-4 cm in length often found fallen on the ground. Tender Coconut Water, a popular folklore oral fluid and electrolyte supplement was selected to see if it has an astringent activity that contributes to its antidiarrheal properties. In addition, three plant based herbal formulations studied were the Link Sudantha toothpaste (Manufactured by Link Natural Product (Pvt) Ltd, No, 11680, Kapugoda, Dompe), Lever Ayush toothpaste (Manufactured by Unilever Sri Lanka Ltd, 258, Vincent Perera Mawatha, Colombo 14) and Link Samahan sachet, containing granules for reconstitution for oral administration (Manufactured by Link Natural Product (Pvt) Ltd, No, 11680, Kapugoda, Dompe). To facilitate a comparative assessment of the efficacy, two chemical based marketed products included were, Medix Lacto - Calamine powder (Manufactured by Medix Pharmaceutical Industrial, Kadawatha, Sri Lanka) and Signal toothpaste (Manufactured by Unilever Sri Lanka Ltd, 258, Vincent Perera Mawatha, Colombo 14). The present study was undertaken to confirm and expand the findings of a previous study in the same laboratory by including new plant materials and formulated preparations. (22)

Neem (*Azadirachta indica*) samples were authenticated by Bandaranaike Memorial Ayurvedic Research Institute. (Account no: 2082). Mature neem leaves were finely

chopped and used. "*Perumkayam*" (Asafoetida powder) was purchased from "*Wijerama Hela Osu Hala and Weda Gedara*", and Roasted spice was purchased from the local market. Very tender coconuts or *Gobalu* (Typica Tall Variety) were obtained from the Naiwala area, Veyangoda. Tender stalk ends of very tender coconuts were ground in to into a paste for testing purposes. Formulations tested included three toothpastes (Link Sudantha toothpaste, and Lever Ayush toothpaste) all of which are herbal toothpastes. One dusting powder, Lacto-Calamine Powder, and the flue medicine Link Samahan Sachet was also tested. Tannic Acid (ACS reagent, brand Sigma – Aldrich) was used as reference study material.

Instruments used in the study were electronic balance (CAS Corporation, Model MWP-600H, 2014, Japan), analytical balance, (OHAUS Corporation, Model PA214C, 2015, USA), UV spectrometer (Agilent Technologies, Model G6860A Cary 60, Malaysia), autoclave (Meditry Instrument Co., Ltd, Model LS-B35L-1, 2014, China), hot-air oven (Memmert GmbH, Model 100D 06057, Germany), incubator (SANYO Electric Co., Ltd, Model MIR-162, Japan) and laminar air cabinet (Jinan Biobase Biotech co., Ltd, Model FH1500, 2014, China). Two pathogenic bacteria used in the *in vitro* antimicrobial assays were *Escherichia coli* (ATCC 25922) and *Salmonella aerues* (ATCC 25923). The microorganisms were obtained from the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura.

Determination of TAAEq values of selected folklore plant materials and formulations

Test procedures were carried out according to a modified protein precipitation assay. (22) Tannic acid standard solution was prepared as follows. Tannic acid 2 g was dissolved in 150 mL of freshly boiled distilled water and allowed to stand for 15 minutes to obtain tannic acid standard solution.

A 2 g quantity of each selected folklore material was boiled in 150 parts (150 mL) of distilled water and allowed to stand for 15 min. The supernatant was collected by decanting. Tender Coconut Water was used without any treatment. For the analysis of commercial formulations, two 2 g portions of each formulation were dissolved in 150 ml of freshly boiled distilled water but without subjecting them to actual boiling.

Egg albumin stock solution was prepared as follows. Colorless egg albumin was collected without mixing egg yolk. Egg white 5 g (*i.e.*, fluid fraction without gel-like strands) was pipetted out by weighing it into a 100 mL beaker. Then 50 mL of purified water was added with gently shaking to homogenize. The albumin stock solution was prepared freshly each time for the study and this solution served as the albumin standard stock solution.

Before carrying out the main protein precipitation assay, a pilot study was done by titrating with Biuret reagent (0.9% w/v sodium potassium tartrate, 0.3% w/v copper sulfate pentahydrate, and 0.5% w/v potassium iodide) to determine the approximate endpoints of test samples. During the main study, on reaching the approximate endpoint, 1 mL aliquots of

aqueous test solutions was added to the egg albumin stock solution until the endpoint was reached. Increasingly smaller amounts of test solution were added when nearing the endpoint. During each addition**,** the reaction mixture was left for 10 minutes with occasional gentle shaking. The endpoint is determined when the natural color of the supernatant of the reaction mixture was barely visible. At the end of 10 minutes following each addition, 2 mL of the supernatants was withdrawn from the reaction mixture, 1 mL was for the Biuret test, other 1 mL was to determine absorption at λ maxima of 280 nm. The endpoint of the protein precipitation assay was determined when the absorption value reached the minimum. Beyond this point, the curve tends to rise again or remain flat. Graphs were constructed using absorption vs. volumes consumed by folk material and formulations. A computer-aided curve was constructed using "Origin Pro" data analysis a graphing software (version 9.1) and the volume consumed at the endpoint was recorded. The same test procedure was repeated for all the astringent materials tested including the tannic acid standard solution, all assays were conducted in triplicate. TAAEq values were determined using the following equation

$$\text{Tannic acid astringent equivalent} = \frac{\text{[Consumed volume of the sample against the egg albumin stock solution]}}{\text{[Consumed volume of the standard tannic acid solution against the egg albumin stock solution]}}$$

According to the above formula mathematically the TAAEq value of the tannic acid standard solution settles as 1. (22)

Preparation of Mueller-Hinton agar medium

All glassware was sterilized in the hot air oven at 160^0C for 1 hour. Mueller-Hinton agar medium powder 9.5 g was transferred to a 300 mL conical flask containing 250 mL of distilled water and dissolved. The agar solution was autoclaved at 15 psi pressure (121^0C) for 30 minutes. The hot agar solution was poured up to 1/3 depth of agar plates and allowed to solidify. All these procedures were carried out in a laminar airflow cabinet under aseptic conditions.

Preparation of bacterial suspension, positive control, negative control, and test samples

An inoculum of 24 hours fresh sub – cultured of *Escherichia coli* (ATCC 25922) and *Salmonella aerues* (ATCC 25923) were taken out using a sterilized wire loop and suspended in 20 mL of sterile normal saline (0.9% w/v sodium chloride solution) separately. The turbidity of each bacterial suspension was compared with the turbidity of McFarland Standard 1 (3×10^8 CFU/mL) solution to achieve desirable cell density.

Sterile distilled water was used as the negative control and tannic acid was selected as the positive control. Tannic acid powder 5 g was dissolved in 20 mL boiled sterile distilled water in a sterile beaker (0.25 g/mL). Of each selected folklore material, infusions were prepared with 5 g quantities and labeled accordingly.

Antimicrobial activity of folklore materials and formulations

Agar disc diffusion assay (24) was carried out to determine the antimicrobial activities of each selected folk material and formulation. Bacterial suspension 1800 µl, were drawn into micropipette and

transferred into solid agar medium. Plates were turned to spread bacterial suspension equally on the surface of the agar plates. Excesses amount of bacterial suspension was drawn back to the micropipette by slightly tilting the plates. Each disc was loaded with 50 µL of the prepared test samples, positive control and negative control. All discs were subjected to air drying under the aseptic conditions.

Then sample loaded discs in triplicate were placed into the labeled agar plates using sterile forceps. Prepared petri dishes were incubated at 37^0C for 24 hours. All the procedures were carried out under aseptic conditions in a laminar airflow cabinet. After 24 hours of incubation, the diameters of inhibition zones in three directions were measured and recorded separately for each folklore material, commercial formulation, and positive control for both bacteria species. Observed zones of inhibitions were measured and standard deviation was calculated.

To obtain the correlation coefficient (r) scatter charts were drawn using TAAEq values of each selected test sample against their inhibition zones for both the selected bacterial species separately.

RESULTS

Graphs were constructed during the protein precipitation assay using absorption vs. end-point volumes consumed by folk material and formulations. The graphs generated for Asafetida and the commercial preparation Link Samahan Toothpaste are depicted in Figures 1 and 2 respectively.

According to the results of the Protein Precipitation Assay, the minimum consumption values of 0.40± 0.1 mL for Neem leaf extract and maximum consumption value of 6.40± 0.31 mL for Roasted Spices were observed (Table 1). For the commercial formulations, the minimum consumption value was seen for Link Sudantha toothpaste (1.5 ± 0.05 mL) and the highest consumption value was seen for Link Samahan sachet (11.7 ± 0.09 mL) (Table 2). In the same two tables to obtain TAAEq values all endpoint values of the samples were divided by endpoint value of the tannic acid standard solution, which was 4.5 mL. Accordingly, the TAAEq value of TASS is 4.5/4.5 = 1.

Using the vernier calipers, diameters were measured for the inhibition zones in three different directions. The mean diameter was taken for each zone for both bacterial species separately.

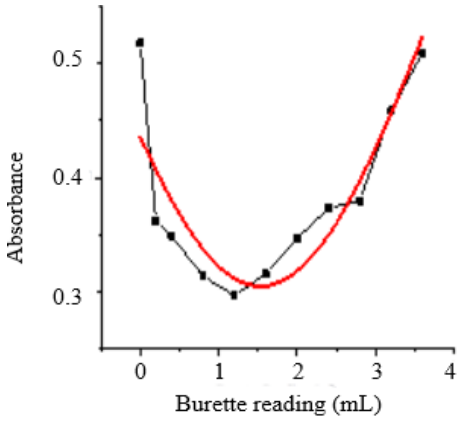

Figure 1. Protein precipitation assay absorption curve for Link Samahan Toothpaste

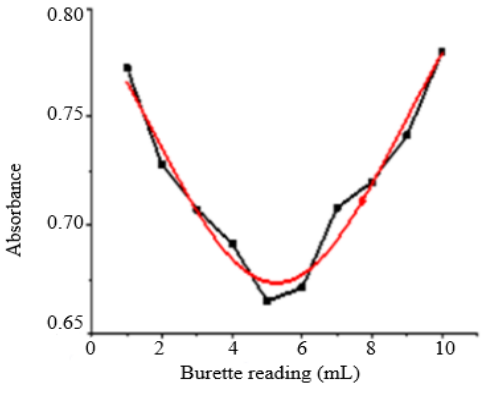

Figure 2: Protein precipitation assay absorption curve for *Perumkayam* (Asafetida)

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Table 1. End point volume in Protein Precipitation Assay and TAAEq values of folklore materials

Folklore Material	End Point (ml)	TAAEq (Sample volume/Tannic acid volume)
Neem Leaves	0.4±0.10	0.09
Tannic acid standard solution	4.5±0.00	1.00
Very tender coconut (*Gobalu*)	5.1±0.35	1.13
Asafetida	5.4±0.99	1.20
Tender coconut water	6.0±1.02	1.33
Roasted spice	6.4±0.31	1.42

TAAEq: Tannic acid astringent equivalent.

Table 2. End Point volume in Protein Precipitation Assay and TAAEq value of formulations

Formulation	End Point (ml)	TAAEq (Sample volume/Tannic acid volume)
Link Sudantha toothpaste	1.5±0.05	0.33
Lever Ayush toothpaste	2.3±0.18	0.51
Tannic acid standard solution	4.5±0.00	1.00
Medix Calamine powder	7.1±0.09	1.57
Signal toothpaste	7.4 ± 0.27	1.64
Link Samahan	11.7 ± 0.09	2.60

TAAEq: Tannic acid astringent equivalent. (Standard Deviation)

According to the data; the maximum inhibition zone was seen for the positive control tannic acid for both bacteria, *S. aureus* 17.8 ± 0.3 mm and *E. coli* 15.1 ± 0.2 mm (Table 3). Among the folklore materials, the maximum inhibition zone was observed for neem leaves for both bacteria, *S. aureus* 12.1 ± 0.05 mm and *E. coli* 12.5 ± 0.87 mm. The minimum was observed for Roasted Spices for both bacteria, *S. aureus* 7.1 ± 0.09 mm and *E. coli* 9.3 ± 0.33 mm. Discs containing tender coconut water did not show any trace of inhibition. Maximum inhibition zone among test formulations was observed for Link Sudantha toothpaste for both bacteria, *S. aureus* 14.6 ± 0.2 mm and *E.coli* 14.1 ± 0.3 mm. Minimum inhibition zone was seen for Link Samahan infusion for both bacteria, *S.aureus* 6.5 ± 0.4 mm and *E.coli* 0.0 mm indicating that this preparation lacks antimicrobial properties (Table 4). The product rightfully does not claim any such activity. Correlation coefficients between TAAEq vs inhibition zone diameters were determined using Microsoft Office Excel 2010. Results obtained for *S. aureus* and *E. coli* for folklore materials and for the commercial formulations is presented in Table 5.

Table 3. Inhibition zone diameters observed of selected folklore materials for *Staphylococcus aureus* and *Escherichia coli*

Folklore Materials	TAAEq value	*S. aureus* inhibition zone diameter (mm)	*E. coli* inhibition zone diameter (mm)
Neem leaves	0.09	12.1 ± 0.1	12.5 ± 0.9
Tannic acid standard solution	1.00	17.8 ± 0.3	15.1 ± 0.2
Very tender coconut (*Gobalu*)	1.13	9.8± 0.9	11.7± 0.2
Asafetida	1.20	8.8± 0.1	10.8± 0.4
Tender coconut water	1.33	-	-
Roasted spices	1.42	7.1± 0.1	9.3± 0.3

TAAEq: Tannic acid astringent equivalent

Table 4. Inhibition zone diameters observed of selected formulations for *Staphylococcus aureus* and *Escherichia coli*

Formulation	TAAEq value	*S. aureus* inhibition zone diameter (mm)	*E. coli* inhibition zone diameter (mm)
Link Sudantha toothpaste	0.33	14.6 ± 0.2	14.1 ± 0.3
Lever Ayush toothpaste	0.51	12.6 ± 0.3	13.1 ± 0.5
Calamine Powder	1.57	9.1 ± 0.8	8.5 ± 0.2
Signal toothpaste	1.64	9.5 ± 0.5	12.6 ± 0.1
Link Samahan	2.60	6.5 ± 0.4	-

TAAEq: Tannic acid astringent equivalent

Table 5. Correlation coefficient of TAAEq vs microbial inhibition zone diameter

Category of material tested	Correlation coefficient for *S. aureus* (r)	Correlation coefficient for *E. coli* (r)
Folklore plant materials	r = -0.937	r = -0.825
Formulations	r = -0.982	r = -0.877

DISCUSSION

The aim of the current study was to find the scientific basis behind the use of folklore plant medicines in Sri Lanka and collect evidences on the correlation between microbial inhibitory activity and TAAEq values of the study plant materials. The astringent activity of the standard extract of each material is the 'Astringent Power' represented by the protein precipitation assay end point volumes. When this activity is related to that of standard tannic acid solution, (TAAEq) is arrived at. When TAAEq values are listed in a given order where the 'TAAEq Index' is arrived at. According to the data astringent activity increases in the order, roasted spices mixture < tender coconut water < Asafetida powder < very tender coconut (Sinhala, *Gobalu*) < neem leaves for the selected

folklore materials studied. The anti-microbial activity increases in the order roasted spices mixture < Asafetida powder < very tender coconuts (Sinhala, *Gobalu*) and < neem leaves for both bacterial species studied.

The increasing astringent activity of the formulations is in the order, Link Samahan < Signal toothpaste < Calamine powder < Lever Ayush toothpaste and < Link Sudantha toothpaste. The antimicrobial activity increases for both study bacteria in the order, Link Samahan < Lacto-Calamine powder < Signal Toothpaste < Lever Ayush toothpaste and < Link Sudantha toothpaste. According to the data, it was clear that both folklore materials and formulations with maximum astringent activity also possessed maximum antimicrobial activity. Furthermore, both herbal toothpaste products Link Sudantha and Lever Ayush showed high astringent activity and high antimicrobial activity compared to the chemical formulation of Signal toothpaste.

It is noteworthy that, although tender coconut water achieved higher astringent activity than roasted spices there was no zone of inhibition observed. Tender coconut water (coconut liquid endosperm) is a growth supplement and it is a good media for the development of microbes. In addition it reduces the lag phase and enhance the log phase of the growth curve of different microbes including *E.coli* and *S. aureus*.(25) Hence a zone of inhibition was not observed with the tender coconut water. (26) During the analysis of commercial products, it was observed that, although Calamine Powder achieved higher astringent activity than Signal toothpaste, it possessed low antimicrobial activity compared to the toothpaste. (27)

The test methods are susceptible to biological variations of the materials used in the study. For greater accuracy the amount of egg albumen used for the standard solution must be based on the protein content and the tannic acid used must be of the given specification for all studies since some of the related compounds too are referred to as tannic acid. Between the two antimicrobial activity analysis procedures of determining the length of the inhibition zone and colony count measurements, the more appropriate procedure needs to be identified. (22)

An event of singular significance in the study was that the only orphaned part thus far evading any modern use or terminology related to the coconut palm was restored to its rightful place. *Gobalu* in Sinhala was named as ‘very tender coconut’. This refers to the youngest coconuts 3-4 cm in length often found fallen on the ground. The paste or a formulation made with the very tender coconuts may have the potential for tanning live skin and could be employed in masking vitiligo patches.

CONCLUSION

Tannic acid astringent equivalents and antimicrobial activity of selected folklore plant materials and selected formulations available in the market were performed as accurately with the aid of biological assay method. The correlation coefficient (r) antimicrobial activity against TAAEq for folklore plant materials for S. *aureus* is -0.937 and against *E. coli* is -0.825. The Correlation coefficient (r) for antimicrobial activity against TAAEq for formulations against *S. aureus* is -0.9825 and against *E. coli* is -0.8774 demonstrating highly negative correlation. (28) The results of the current study revealed that the tested materials which possess high composite astringent activity also possessed high

antimicrobial activity. Therefore, this study proved the scientific basis of the use of the studied folklore medicines by traditional Sri Lankan society for thousands of years for the cure of diseases using scientific techniques.

The correlation between antimicrobial activity and astringent activity was observed. TAAEq values and antimicrobial activities of many other folklore medicines and chemical substances can be investigated based on the procedures set out in this study. In same plant TAAEq values and antimicrobial activities for different growth stages, parts, geographical regions and different seasons can be investigated. TAAEq values could be displayed in applicable product labels for consumer information along with other indicators such as glycaemic value. If marketed formulations can indicate both astringent activity and antimicrobial activity it would be very useful to the healthcare personnel and the customers who purchase those formulations over-the-counter. TAAEq values and antimicrobial activities may be established as specifications for applicable herbal products. By collecting TAAEq values of plant materials and marketed formulations, a comprehensive "Astringent Index" for each category could be developed.

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Author's declaration:
The authors declare that all persons listed as authors have read and given approval for the submission of this manuscript.

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

REFERENCES

1. Peña-Neira A. Management of astringency in Red Wines. Red Wine Technology. 2019;257–72.
2. Ashok PK, Upadhyaya K. Tannins are Astringent. J Pharmacogn Phytochem. 2012;1(3):45–50.
3. Field JA, Lettinga G. Toxicity of tannic compounds to microorganisms. In: Hemingway RW, Laks PE, editors. Plant Polyphenols. Synthesis, Properties, Significance.. New York: Springer S; 1992. p. 673-692.
4. Farha AK, Yang QQ, Kim G, Li HB, Zhu F, Liu HY, et al. Tannins as an alternative to antibiotics. Food Bioscience. 2020;38:100751.
5. Tomiyama K, Mukai Y, Saito M, Watanabe K, Kumada H, Nihei T, *et al*. Antibacterial Action of a Condensed Tannin Extracted from Astringent Persimmon as a Component of Food Addictive Pancil PS-M on Oral Polymicrobial Biofilms. Biomed Research International. 2016;2016.
6. Padmasiri GR. An investigation into utilization, beliefs and practice of indigenous medicine in Sri Lanka. African Journal of Traditional, Complementary and Alternative Medicines. 2018;15(4): 1–12.
7. Gamage DG, Dharmadasa RM, Abeysinghe DC, Wijesekara RG, Prathapasinghe GA, Someya T. Ethnopharmacological Survey on Medicinal Plants Used for Cosmetic Treatments in Traditional and Ayurveda Systems of Medicine in Sri Lanka. Evidence-Based Complementary and Alternative Medicine. 2021 Jun 28;2021

8. Houghton PJ. The Role of Plants in Traditional Medicine. Journal of Alternative and Complementary Medicine. 1995;1(2):131–43.
9. Lohiya NK, Balasubramanian K, Ansari AS. Indian folklore medicine in managing men's health and wellness. Andrologia. 2016; 48(8):894-907.
10. Rangasamy O, Raoelison G, Rakotoniriana FE, Cheuk K, Urverg-ratsimamanga S, Quetin-leclercq J, et al. Screening for anti-infective properties of several medicinal plants of the Mauritians flora. Journal of Ethnopharmacology. 2007; 109(2):331-7.
11. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research. 2011;5(31):6697–703.
12. Pathogens I. Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive Compounds against Clinically Important Pathogens. Antibiotics. 2019;8(4):257.
13. Sharma A, Patel VK, Chaturvedi AN. Vibriocidal activity of certain medicinal plants used in Indian folklore medicine by tribals of Mahakoshal region of central India. Indian journal of pharmacology. 2009;41(3):129–34.
14. Darwish RM, Aburjai TA. Effect of Ethnomedicinal Plants Used in Folklore Medicine in Jordan as Antibiotic Resistant Inhibitors on Escherichia coli. BMC Complement Altern Med. 2010; 10(1):1–8.
15. Chaudhri SK, Jain NK. History of Cosmetics. Asian Journal of Pharmaceutics. 2014; 3(3):164–167.
16. Parish LC, Crissey JT. Cosmetics: A Historical Review. Clinics in Dermatology. 1988; 6(3):1-4.
17. Ashawat M, Banchhor M, Saraf S, Saraf S. Herbal Cosmetics:" Trends in Skin Care Formulation". Pharmacognosy Reviews. 2009; 3(5): 82–9.
18. Gautam B, Dongol E, Shrestha A. *In-vitro* antimicrobial activity of different toothpastes. Journal of Kathmandu Medical College. 2017;6(2):52-8.
19. Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, et al. Antimicrobial activity of the essential oil from Lippia sidoides, carvacrol and thymol against oral pathogens. Brazilian Journal of Medical and Biological Research. 2007; 40(3):349-56.
20. Huynh Q, Phan TD, Thieu VQQ, Tran ST, Do SH. Extraction and refining of essential oil from Australian tea tree, Melaleuca alterfornia, and the antimicrobial activity in cosmetic products. J. Phys. Conf. Ser. 2012, 352, 012053.
21. Alzomor AK, Moharram AS, Al Absi NM. Formulation and evaluation of potash alum as deodorant lotion and after shaving astringent as cream and gel. Int Curr Pharm J. 2014;3(2):228–33.
22. Wagarachchi AN, Dilshan WG, Pathirana W, Siriwardhene MA, Obeysekera C. Composite Astringent Index and the Determination of Anti-infective Properties of Selected Folklore Therapies. Pharmaceutical Journal of Sri Lanka. 2020;10(1):64-73.
23. Ediriweera ERHS. Medicinal uses of coconut (Cocos nucifera L.). Coco info Int. 2003;10:11–21.
24. De Silva RT, Dissanayake RK, Mantilaka MMMGPG, Wijesinghe WPSL, Kaleel SS, Premachandra TN, et al. Drug-loaded halloysite nanotube-reinforced electrospun alginate-based nanofibrous scaffolds with sustained antimicrobial protection. ACS applied materials & interfaces. 2018;10(40):33913–22.

25. Prades A, Dornier M, Diop N, Pain JP. Coconut Water Uses, Composition and Properties: A review. Fruits. 2012;67(2):87–107.

26. Rukmini JN, Manasa S, Rohini C, Sireesha LP, Ritu S, Umashankar GK. Antibacterial Efficacy of Tender Coconut Water (Cocos nucifera L) on Streptococcus mutans: An In-vitro Study. Journal of International Society of Preventive & Community Dentistry. 2017; 7(2): 130–5.

27. Roopavathi KM, Gopal SV, Pushpalatha G, Antharasanahalli DB, Madhura S. Antimicrobial Efficacy of Commercially Available Toothpastes-An In vitro Study. Journal of Young Pharmacists. 2015;7(3):187–93

28. Ratner B. "The correlation coefficient: its values range between + 1 / − 1, or do they?". Journal of targeting, measurement and analysis for marketing. 2009; 17(2):139–42.