Journal of Traditional and Complementary Medicine Vo1. 4, No. 3, pp. 196-202

Journal of Traditional and Complementary Medicine
Journal homepage http://www.jtcm.org

The Classification of Sri Lankan Medicinal Herbs: An Extensive Comparison of the Antioxidant Activities

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ABSTRACT

Sri Lanka has variety of herbs whose effectiveness has been proven across many generations. These herbs are classified into two groups — 'heating' and 'cooling', based on the physiological reactions upon consumption. Application-wise, the 'cooling' herbs are administered to patients contracted with diabetes, imbalances in the lipid profile, or even cancer. However, this classification has been misunderstood due to inconsistent interpretations and lack of scientific reasoning. This study systematically determines the rationale behind this classification, by specifically evaluating the antioxidant activity of 18 herbs — nine herbs from each category. The oxygen radical absorbance capacities, DPPH radical scavenging activities, and the total phenolic contents are analyzed here. The 'heating' herbs have a comparatively lower antioxidant potential than the 'cooling' herbs. The total phenolic contents correlate with the antioxidant values. It can be hypothesized that the high antioxidant potential of the 'cooling' herbs may have been responsible for the containment of the diseases mentioned previously.

Key words: Antioxidant, Di (phenyl)-(2, 4, 6-trinitrophenyl) Iminoazanium (DPPH), Oxygen radical absorbance capacity

INTRODUCTION

Located in the tropics, Sri Lanka has an assortment of plant species that have been consumed for generations as herbal treatments, for control of diseases. Some of the diseases with complicated etiologies such as diabetes, arthritis, and cancer (for which a permanent cure is not in sight at present) have been known to be completely controlled or cured using these herbal remedies alone.[1] This traditional medicinal system, which has more than 3000 years of tested and proven efficacy, is still in use and generally the first approach for disease control by the locals, especially those who have been contracted with the stated diseases.[2] Typically, the herbs being used for medicinal purposes are evergreen in nature and are grown in the backyards of houses, and very little nurturing effort is required for their growth. Some of these herbs are even considered as weeds due to their high growth rates. Most Sri Lankans are familiar with the traditional medicinal system and are even able to identify or administer the herbs growing within their area of residence. Thus, the locals can be observed consuming these herbs to control a disease without the advice of a traditional medicinal practitioner, as they are familiar with the usage of these herbs because of the traditional knowledge, which has been passed down by their ancestors.[3]

Heat — or an element related to heat—is a feature that has been identified in many traditional medicinal systems as an essential characteristic for maintaining physical equilibrium. In Traditional Chinese Medicine (TCM) for instance, *yin* and *yang* are used to express the dual opposite qualities of human physiology.[4] The counteractive properties of medicines attributed to water, coldness, and darkness are classified under *yin*, while those that

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DOI: 10.4103/2225-4110.126175

inculcate properties such as fire and brightness are associated with *yang*.[5] Maintaining the *yin* and *yang* in harmony has been equated with attaining physical homeostasis. In a similar fashion, Indian Ayurvedic Medicine stresses on a balance of three elemental substances associated with heat for achieving physical equilibrium: They are, *āyu* or *vāta* (air/space/wind), *pitta* (fire), and *kapha* (water/earth).[6] According to this medicinal system, every individual has an innate combination of these three elements, for which the relevant balance requires to be pursued by appropriately structuring their behavior or environment. The Sri Lankan traditional medicinal system (which is also known as 'Ayurveda') — a mixture of the Sinhala traditional medicine, Ayurveda, and Siddha systems of India, and the Unani medicine of Greece, through the Arabs — similarly considers components related to heat, when defining the maintenance of health and wellness.[7] On account of the influence of Indian Ayurveda, the elements of *vāta*, *pitta,* and *kapha* remain unchanged when defining diseases in the Sri Lankan traditional medicinal system. However, the two schemes go separate ways when it comes to the treatment methods, in that, the herbs used for the same diseases tend to be different, mostly owing to the matter of availability.

The herbs used in the Sri Lankan traditional medicinal system are broadly classified as 'heating' and 'cooling' herbs.[8] Distribution of herbs into either of the groups is based on the physiological reactions upon consumption, considering the balance/imbalance of *vāta*, *pitta,* and *kapha.*[7] According to the traditional definitions, the 'cooling' herbs tend to aggravate phlegm and increase the oozing sensations of the body (i.e. adding more *vāta* or *kapha*), while the 'heating' herbs tend to increase inflammatory situations (i.e. adding more *pitta*).[9] Thus, the traditional rationale behind the application of 'heating' herbs is to increase the *vāta* or *kapha*, while the 'cooling' herbs are used for diseases requiring *pitta*. It has to be borne in mind, nevertheless, that similar to the *yin* and *yang* of TCM, *vāta*, *pitta,* and *kapha* — regardless of whether it is in the Indian or Sri Lankan medicinal system, does not have any concrete physical meaning within the modern scientific scope, and thus, there is no equivalent term in western medicine to aptly describe these elements.

Modern western medicine considers the balance between oxidation and antioxidation as a critical concept for maintaining a healthy biological system.[10] For instance, oxidative stress is defined as a condition where the oxidative reactions are in excess and is considered as the root cause of disease conditions such as diabetes.[11] As a result, antioxidants have been touted as potential remedies for the long-term complications of diabetes or diseases of a similar magnitude.[11,12] In the Sri Lankan traditional medicinal system, diabetes has been defined as a disease that is caused by the overreactions of *vāta* and *kapha* and/or the absence or decrease of *pitta.*[13] Thus, the 'cooling' herbs are mostly administered to diabetic patients. For the purpose of adding a scientific perspective, whether the 'cooling' herbs contain antioxidant potential is worthy of investigation, as in a similar context, a comprehensive study had been conducted on finding the correlation between *yin–yang* and antioxidant–oxidant properties, where a clear trend was observed between the two categories of herbs.[14] Thus, the objective of this study was to examine whether the 'cooling' and 'heating' herbs of Sri Lanka were correlated with the antioxidant–oxidant characteristics. This study, the first of its kind, serves as a stepping stone for elucidating and characterizing the antioxidant potential of herbs commonly used in the Sri Lankan Traditional Medicinal Pharmacopoeia. Given that the Sri Lankan Traditional Medicinal System has not received the same amount of attention as TCM or Indian Ayurveda, scientific research has not been conducted at all on some of the herbs examined in this study. Thus, results from this study showcase unexplored territories for future studies to focus on, which will have a significant bearing on discovering novel therapeutics with the capabilities of remedying global pandemics such as diabetes, cardiovascular disease, and cancer.

METHODS

All chemicals used for this study were purchased from Sigma-Aldrich (Bangalore, India), Fluka (USA) or Sigma Chemicals (USA), unless otherwise specified.

Preparation of herbal extracts

Eighteen authentic and representative medicinal herbs [Table 1] were chosen according to their properties, as documented in the authoritative literature.[15,16] Nine herbs were selected from each category. Only leafy herbs were chosen, as the leaf reportedly contains the highest amount of antioxidant compound compared with the bark.[17,18] It also needs to be emphasized that these leaves were consumed for therapeutic purposes by traditional practice. Leaves from the aerial part of the plants were collected from the central province of Sri Lanka and identified by comparison with the respective Herbarium specimen available at the National Herbarium of the Peradeniya Botanical Gardens in Kandy, Sri Lanka. A voucher specimen was deposited at the Institute of Fundamental Studies, Hantane Road, Kandy, Sri Lanka. The herbs were intensively sun-dried for eight hours and ground into a powder. Twenty milliliters of water at 60°C was added to one gram each of the powders to prepare a decoction, which was allowed to cool to room temperature at 25°C. The temperature of 60°C was used, as the traditional preparation of the selected herbs did not engage a very high temperature. The mixtures were centrifuged at 1000 rpm and the supernatants were separated, freeze-dried, and stored at −20°C till the analyses were carried out. Working solutions of herbal extracts of 1000, 500, 250, 125, and 62.5 ppm were prepared for all assays.

Oxygen radical absorbance capacity assay

The Oxygen Radical Absorbance Capacity (ORAC) value of the herbal extracts was analyzed according to the method of Huang, Ou, Flanagan, and Deemer.[19] Briefly, the antioxidant capacity of the herbal extracts was measured in terms of trolox equivalents (TE). Vitamin C was used as a positive control. Fluorescein disodium was used for the kinetic monitoring of free radical quenching and 2, 2-Azobis (2-amidinopropane) dihydrochloride (AAPH) was used as the free radical source. The assay was carried out in a 96-well microplate format using the Thermo Scientific Multiskan FC Microplate Reader. The excitation and emission wavelengths were 485 nm and 528-538 nm,

Table 1. Botanical, family, and vernacular (Sinhala) names of the herbs selected for the study and their representative images

Botanical name	**Family**	**Vernacular name**	**Image of the herb**
Adhathoda vasica	Acanthaceae	Adhathoda	
Amaranthus viridis	Amaranthaceae	Kura Thampala	
Alternanthera sessilis (蓮子草 Lián Zǐ Cǎo)	Amaranthaceae	Mukunuwenna	
Annona muricata	Annonaceae	Katu Anoda	
Artocarpus heterophyllus	Moraceae	Kos	
Asparagus racemosus	Asparagaceae	Hathawariya	
Centella asiatica (積雪草 Jī Xuě Cǎo)	Mackinlayaceae	Gotu kola	
Coccinia grandis	Cucurbitaceae	Kowakka	
Costus speciosus	Zingiberaceae	Thebu	
Desmodium gangeticum	Fabaceae	Udupiyaliya	
Gymnema sylvestre	Asclepiadaceae	Masbadda	

Contd....

Table 1. Contd....

Botanical name	**Family**	**Vernacular name**	**Image of the herb**
Ipomoea aquatica (蕹菜 Wèng Cài)	Convolvulaceae	Kangkung	
Mimosa pudica (含羞草 Hán Xiū Cǎo)	Fabaceae	Nidikumba	
Momordica charantia (苦瓜 Kǔ Guā)	Cucurbitaceae	Karavila	
Psidium guava	Myrtaceae	Pera	
Sesbania grandiflora	Fabaceae	Kathurumurunga	
Solanum americanum	Solanaceae	Kalukammeriya	
Wattakaka volubilis	Asclepiadaceae	Kiri Anguna	

respectively. The following components were added to a single well: (1) Blank (phosphate buffered saline)/trolox standard/sample – 20 μL, (2) fluorescein working solution – 160 μL, and (3) AAPH – 20 μL. The reaction kinetics were monitored for two hours at 37ºC, following which the area under the curve was used to calculate the ORAC value compared with those of the trolox standards. Results were expressed as μmol TE per gram of herbal extract.

Determination of the Di (phenyl)-(2, 4, 6-trinitrophenyl) iminoazanium radical scavenging activity

Extracts of the herbs at concentrations of 1000, 500, 250, 125, and 62.5 ppm were prepared by dilution with 75 mM phosphate buffer (pH = 7.40). A 96-well microplate was used for the analysis where 140 μL of the extracts of the herbs were pipetted along with 60 μL of 400 μM of DPPH (prepared in the phosphate buffer solution). The blank wells consisted of 200 μL of the phosphate buffer solution, while the control wells consisted of 140 μL of the phosphate buffer solution and 60 μL of the DPPH solution. The microplate was incubated at 37ºC for 30 minutes and the absorbance was measured at 517 nm, using a Thermo Scientific Multiskan FC Microplate Reader. Each sample concentration was added in triplicate into the microplate. Vitamin C was used as the positive control. The antioxidant activity was calculated as % DPPH radical scavenging activity, by substituting the absorbance values into the following equation:

$$\%\,\text{DPPH radical scavenging activity} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Extract}}}{\text{Abs}_{\text{Control}}} \times 100$$

The % DPPH scavenging activity of 10 replicates of each sample was used to calculate the EC_{50} values (in ppm) of the individual herbs and their combinations.

Determination of the total phenolic content

The method, as described by Singleton and Rossi, was used for determining the total phenolic content of the extracts of the herbs.[20] However, the assay was carried out in the 96-well microplate format. Gallic acid was used as the standard to plot a curve where concentrations of 50.0, 25.0, 12.5, 6.2, and 3.1 mg/mL were prepared by carrying out serial dilutions using deionized (DI) water. The following constituent volumes were added to a single well: Folin-Ciocalteu reagent – 100 µL, sample/blank (DI water)/gallic acid standard – 20 µL, Na_2CO_3 (30g/L) – 80 µL. The microplate was incubated at room temperature for 15 minutes, following which, the absorbance was read at 540 nm by using the Thermo Scientific Multiskan FC Microplate Reader. Dilutions were performed on the herbal extracts as deemed necessary, for the absorbance values to fit within the gallic acid standard curve.

Statistical analysis

For calculation of the EC_{50} values of the DPPH radical scavenging activity of the herbs, the IBM SPSS Statistics version 21.0 (released on August 2012) for Windows was used. The results were calculated and expressed as mean ± SEM of ≥ 3 independent analyses.

RESULTS

As shown in Table 2, the antioxidant activities of the 'cooling' herbs had the highest ORAC values and DPPH radical scavenging values (EC_{50}), ranging from 1096.2-1983.6 µmol TE/g to 90.3 – 258.3 ppm, respectively. Some of the popular 'cooling' herbs that have been used, in particular, for diabetes, such as, *Coccinia grandis* and *Costus speciosus,* had ORAC and DPPH EC_{50}values comparable with vitamin C (2000 µmol TE/g and 81.3 ppm, respectively). The 'heating' herbs had comparatively less ORAC and DPPH radical scavenging values, demonstrating that the 'heating' property of these herbs was unlikely to be related to their antioxidant activity. It was very unlikely that the effective components of the 'heating' herbs were simply oxidants. Nevertheless, it will be of interest to know the mechanism of action of the phytochemicals in these herbs, in achieving their therapeutic potential.

From Table 1, it is also evident that the 'cooling' herbs contain a comparatively higher total of phenolic level than the 'heating' herbs. In comparing the correlation between the ORAC and DPPH EC_{50}values versus the total phenolic contents, it has been observed that the ORAC values have a better linear response compared to the EC_{50} values, although the linearity of the ORAC values is not perfect [Figure 1]. This may be because the method used for total phenolic content is structurally insensitive, whereas, the ORAC value is structurally sensitive.[21,22] Therefore, the quantity of phenolic compounds in a sample may not necessarily have a linear correlation with its antioxidant activity, regardless of the assay method.

DISCUSSION

One of a few stable and commercially available organic nitrogen radicals is DPPH, where the basis of the kinetic analysis is the reaction between phenols and DPPH.[23] For the ORAC assay, a thermal radical generator is applied to give a steady flux of peroxyl radicals in an air-saturated solution. The antioxidant that is present in the sample or standard competes with a fluorescent probe for the radicals and inhibits or retards the probe oxidation.[23] Given the two different approaches, both the assays were carried out in the study to cover all possible reaction mechanisms of the

Table 2. Antioxidant activity and total phenolics (in descending order) of the herbs in the 'heating' and 'cooling' categories

Botanical name	Vernacular name	Family name	ORAC (µmol TE/g)*	DPPH (EC_{50}) (ppm)*	Total phenolics (mg GAE/g)*
'Cooling' herbs					
Coccinia grandis	Kowakka	Cucurbitaceae	1983.6±20.6	90.3±7.6	124.3±10.1
Asparagus racemosus	Hathawariya	Asparagaceae	1823.4±19.4	91.4±6.7	103.2±9.5
Costus specious	Thebu	Zingiberaceae	1743.2±17.5	100.2±9.8	98.3±7.1
Amaranthus viridis	Kura Thampala	Amaranthaceae	1658.7±19.5	96.5±7.5	85.4±6.1
Annona muricata	Katu Anoda	Annonaceae	1544.2±15.6	97.2±6.5	84.3±5.4
Sesbania grandiflora	Kathurumurunga	Fabaceae	1498.6±16.4	126.8±7.1	81.6±6.1
Desmodium gangeticum	Udupiyaliya	Fabaceae	1329.5±17.8	153.4±6.8	80.1±5.3
Mimosa pudica (含羞草 Hán Xiū Cǎo)	Nidikumba	Fabaceae	1182.9±12.5	254.3±9.1	69.2±5.7
Momordica charantia (苦瓜 Kǔ Guā)	Karavila	Cucurbitaceae	1096.2±16.5	258.3±10.1	68.2±4.8
'Heating' herbs					
Alternanthera sessilis (蓮子草 Lián Zǐ Cǎo)	Mukunuwenna	Amaranthaceae	597.1±10.5	315.3±11.4	55.3±3.7
Artocarpus heterophyllus	Kos	Moraceae	593.2±11.4	315.2±12.6	54.3±3.5
Adhathoda vasica	Adhathoda	Acanthaceae	553.8±12.1	316.4±11.8	51.8±3.5
Psidium guava	Pera	Myrtaceae	520.2±12.5	302.1±12.1	52.4±3.1
Solanum americanum	Kalukammeriya	Solanaceae	483.9±10.1	348.9±13.5	49.6±2.9
Gymnema sylvestre	Masbadda	Asclepiadaceae	402.1±10.2	417.6±13.2	43.5±2.9
Centella asiatica (積雪草 Jī Xuě Cǎo)	Gotu Kola	Mackinlayaceae	354.2±9.7	504.9±15.4	39.2±2.8
Wattakaka volubilis	Kiri Anguna	Asclepiadaceae	315.3±10.1	506.1±14.9	37.6±2.6
Ipomoea aquatica (蕹菜 Wèng Cài)	Kang Kung	Convolvulaceae	298.7±9.7	505.3±17.2	32.5±32.5

ORAC: Oxygen radical absorbance capacity; DPPH: Di (phenyl)-(2, 4, 6-trinitrophenyl); GAE: Gallic acid equivalents; EC: Effective concentration

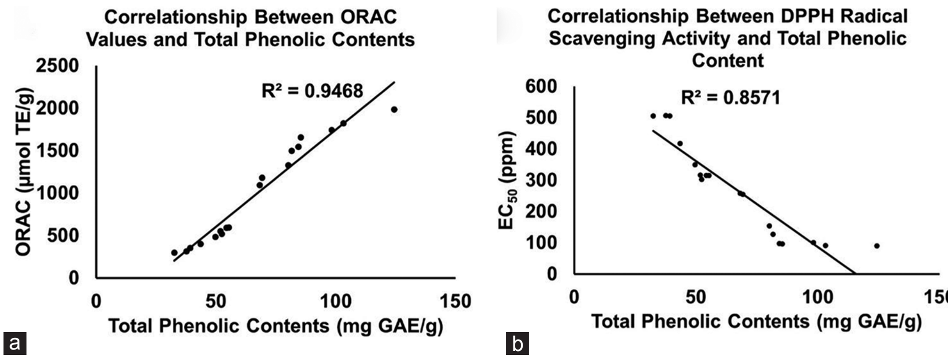

Figure 1. Correlation between ORAC values, DPPH radical scavenging activities (EC50) and the total phenolic contents of the herbs

antioxidants. However, the ORAC values may have had a better correlation with the total phenolic contents, as the phenolic compounds present in the herbal extracts may have been better scavengers of peroxyl radicals.[24,25]

The phenolic compounds are responsible for most of the reported antioxidant activities in plants.[26,27] Although studies have not been carried out to date, to elucidate the effective compositions of the 'cooling' herbs, in this study, it is possible that many flavonoids exist in the extracts, which are phenolic compounds with a strong antioxidant activity. In terms of flavonoids, many of them are known to have anti-inflammatory properties.[28] According to the Sri Lankan medicinal system, inflammation is regarded as a typical physiological symptom of heat.[7] Thus, although the scientific rationale identified in this study was not known by the traditional medicinal practitioners of ancient times, it is possible that through mere observation of the reduction of disease symptoms, the herbs responsible for the therapeutic effect were correctly sorted into the appropriate classification.

The traditional medicinal system of Sri Lanka has not been extensively studied by using modern science. There have been no studies to date, which have been targeted at shedding some light on the chemical nature of the notion of 'heat' and its related concepts used in this ancient medicinal system. The clear trend of the antioxidant activities is supported the hypothesis that 'cooling' or the removal of excess *vāta* and/or *kapha* (or possibly the addition of *pitta*) refers to antioxidation, whereas 'heating' or the removal of excess *pitta* (or possibly the addition of *vāta* and/or *kapha*) refers to oxidation. This hypothesis opens a new avenue for using modern science to study theories that have been in practice in Sri Lanka for over 3000 years. In addition, the empirical observations accumulated over many generations could also be used as learning points for present-day applications, as many of the current global pandemics do not have therapeutic solutions from western medicine. For instance, *Coccinia grandis* and *Costus speciosus* are popular home-remedies for diabetes, which can be easily grown, for supporting cases of patients having hypoglycemic effects.[24] Bittergourd (*Momordica charantia*) has been extensively studied for anti-diabetic effects.[28,29] However, it is generally not known that even the leaves of this plant contain anti-diabetic properties. Given the bitter flavor of the vegetable, consumption of its leaves could be more appetizing due to the absence of the bitterness. Although a 'heating' herb, *Centella asiatica* (積雪草 Jī Xuě Cǎo) is regularly consumed by locals in the form of a salad or added to rice porridge.[30] It has been consumed for balancing the lipid profile, generally to reduce Low-density lipoprotein (LDL) cholesterol levels.[30] Its specific mechanism of action has not been identified to date, however, cases have been reported where its consumption has resulted in regaining a healthy lipid profile.[27,31] In similar fashion, all the herbs examined in this study have been used for one therapeutic purpose or for many. Thus, further investigation is required as to their specific mechanisms of action in order to add scientific proof to their efficacy — a characteristic that will be of value when advocating these herbs for global usage.

CONCLUSION

This study was able to provide scientific evidence to the method of classification of herbs in an ancient medicinal system. Despite the comparative lack of fame, the Sri Lankan traditional medicinal system has been able to remedy many diseases for which a permanent cure is not available from the western counterpart. As shown in terms of the antioxidant assays, the herbs used in this medicinal system carry a very high antioxidant potential. The mechanisms of action of these herbs require further study and determination of this aspect will be able to provide better medicines, with multiple therapeutic properties, which will be more effective in curing and controlling diseases that have a significant impact on the quality of life.

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