



# 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.<sup>[1]</sup> 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.<sup>[2]</sup> 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.<sup>[3]</sup>

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.<sup>[4]</sup> The counteractive properties of medicines attributed to water, coldness, and darkness are classified under *yin*, while those that

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inculcate properties such as fire and brightness are associated with *yang*.<sup>[5]</sup> 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).<sup>[6]</sup> 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.<sup>[7]</sup> 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.<sup>[8]</sup> 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*.<sup>[7]</sup> 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*).<sup>[9]</sup> 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.<sup>[10]</sup> 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.<sup>[11]</sup> As a result, antioxidants have been touted as potential remedies for the long-term complications of diabetes or diseases of a similar magnitude.<sup>[11,12]</sup> 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*.<sup>[13]</sup> 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.<sup>[14]</sup> 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.<sup>[15,16]</sup> 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.<sup>[17,18]</sup> 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.<sup>[19]</sup> 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,

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