Indian Journal of Traditional Knowledge
Vol 24(1), January 2025, pp 7-15
DOI: 10.56042/ijtk.v24i1.12234

Assessment of standardization and quality control parameters of a new formulation of *Trijata*; An Ayurveda formula

Swarna D Hapuarachchi[a,*], Dinithi Silva[b], Chamali Madhushika[a] & Nandani D Kodithuwakku[a]

[a]Department of Ayurveda pharmacology and pharmaceutics, Faculty of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka

[b]Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna, Wellamadama, Matara, Sri Lanka

[*]E-mail: dr.sdhapuarachchi@fim.cmb.ac.lk

Received 22 July 2024; revised 09 December 2024; accepted 08 January 2025

Trijata is a polyherbal Ayurveda preparation containing *Twak* (*Cinnamomum verum* syn. *Cinnamomum zeylanicum*), *Ela* (*Elettaria cardamomum*), and *Patra* (*Cinnamomum tamala*) discussed in Ayurveda textbooks, including Bhava Prakash, Kaiyadeva Nighandu and Ayurveda Pharmacopeia, Sri Lanka. This study aimed to develop a novel preparation of *Trijata* incorporating *C. zeylanicum* leave substituting *Cinnamomum tamala*, due to the unavailability of *C. tamala* in Sri Lanka. Furthermore, quality control and standardization parameters were developed for this Novel *Trijata* (NT). The hot and cold aqueous extractions were subjected to qualitative phytochemical screening. Total phenolic, flavonoid contents, and *in-vitro* antioxidant activity were determined using DPPH and ABTS assays. Microbiological limits, heavy metal content, and physicochemical parameters including; the ash content, and extractable matter were determined for quality control and standardization. Preliminary phytochemical analysis revealed the presence of phenols, flavonoids, tannins, alkaloids, saponins, terpenoids, reducing sugars, and cardiac glycosides in NT aqueous extract. The physicochemical parameters including; 4.6±0.2% of total ash, 3.1±0.1% water-soluble ash, and 0.6±0.2% of acid insoluble ash on a dry weight basis were determined. *In-vitro* antioxidant activity as per DPPH and ABTS assays was dose-dependent and the highest activity was obtained with IC_{50} of hot water extract. Extractability was high for hot extraction. NT had high total phenolic and flavonoid content exhibited through promising antioxidant activity. The microbiological limits and heavy metal content were within the standard acceptable limits. The HPTLC profiles of this study would be more helpful to authenticate this product (NT) for drug manufacture and further studies are recommended to evaluate its biological activities for proper indications.

Keywords: Antioxidant, *Cinnamomum zeylanicum* Blume, *Cinnamomum tamala*, *Elettaria cardamomum*, HPTLC, Novel *Trijata*

IPC Code: Int Cl.[25]: A61K 36/00

Trijata is a formula described in Ayurveda authentic text books including Bhava Prakasha, Kaiyadeva Nighantu and Ayurveda Pharmacopeia, Sri Lanka[1-3]. It is composed of an equal amounts of *Twak* bark powder of Ceylon Cinnamon (*Cinnamomum zeylanicum* Blume (syn *Cinnamomum verum* J. Presl), *Ela* (seeds of *Elettaria cardamomum* (L) Maton), and leaves of *Cinnamomum tamala* (Buch.-Ham.Th.G.G.Nees) as *Patra*. It is also used as an herbal remedy in Ayurveda medical system and Traditional medical systems in Sri Lanka. This formulation holds *Vata-Kapha shamaka* and *Pramehahara* (anti-diabetic) medical conditions as per the Ayurveda texts for over 6000 years as supportive ingredients in various Ayurvedic dosage forms[3]. This formula is also enhancing the aroma and it boosts appetite and digestion. According to the Yogarathnakara book[4], *Trijatha* is indicated for many therapeutic effects including mouth disorders, cough, throat disorders and alleviate the vatta kapha doshic conditions[1-3].

Ceylon Cinnamon is the oldest and most frequently consumed spices and is also used as a herbal remedy in Ayurveda and Traditional medical system in Sri Lanka. It holds many medicinal uses as per the Ayurveda texts for over 6000 years encompassing[1-7].

Spices including Ceylon cinnamon*;* are a group of esoteric food adjuncts contributing to the taste and flavor of food, perfumes, and medicinal products over thousands of years due to their specific aroma. Every part of the plant including; leaves, bark, flowers, fruits, and roots has medicinal uses. These plant parts have similar types of hydrocarbons in different

[*]Corresponding author

proportions mainly; cinnamaldehyde in bark, eugenol in leaves, and camphor in roots[8].

Several *in vitro, in vivo* and human studies had proved the potential role of *C. zeylanicum*[9]. Its formulations have been well-documented for their therapeutic potential, as *Vata-Kaphashamaka. C. zeylanicum* is a major constituent in most Ayurveda formulation, included in authentic Ayurveda textbooks. There are 52 formulations described in Sri Lankan Ayurveda Pharmacopeia containing *Twak* bark (Ceylon Cinnamon); out of which *Trijata* powder and *Candraprabhavati* are the most important and popular formulations that are used as the *Pramehahara* (anti-diabetic) drugs in Ayurveda. Interest in *C. zeylanicum* as a potentially useful treatment for type 2 diabetes mellitus (T2DM) began almost 20 years ago[10]. It had stated that an unidentified factor called insulin potentiating factor (IPF) was isolated from *C. zeylanicum* which was involved in the alleviation of the signs and symptoms of diabetes mellitus and other disorders related to insulin resistance. Furthermore, it was proven that both the bark and leaf extracts of *C. zeylanicum* exhibit *in vitro* anti-diabetic, anti-amylase, and anti-cholinesterase activity and its effect on the management of complications of T2DM[11-13].

However, it had also been found that *C. zeylanicum* is ineffective in improving glycemic control in patients with type 1 diabetes (T1DM)[14]. The *in-vivo* and *in-vitro* studies utilizing a small number of experimental animals or healthy human volunteers had shown that the dried *C. zeylanicum* powder and aqueous *C. zeylanicum* extract can improve cellular glucose metabolism. The evidence remains weak since the experiments are not replicated or contradictory. Clinical trials had not been attempted so far to link the clinical outcomes of the presumed mechanism of action, as well. Although the mechanism of action and the indications of *C. zeylanicum* are outlined, the relevance of its mode of action to clinically significant outcomes remain unknown[15]. Furthermore, *C. zeylanicum* is currently being investigated as a potential preventive supplementation and a remedy for insulin resistance, metabolic syndromes, and T2DM. Extensive *in-vitro* evidence had shown that *C. zeylanicum* may prevent and reverse the insulin-signaling impairment in skeletal muscles. Therefore, *C. zeylanicum* is considered both a preventive and therapeutic supplementation for metabolic syndromes, insulin resistance, T2DM, hyperlipidemia, and arthritis.

C. zeylanicum is a well-known medicinally important plant having antioxidant, anti-inflammatory, anti-nociceptive, antidiabetic, antimicrobial, antifungal, nematocidal, insecticidal, larvicidal, antimycotic, anti-gastric ulcer, anti-secretagogue, anticancer, lipid-lowering, wound healing, hepato-protective and cardio-protective activity. *C. zeylanicum* also holds a protective potential against neurological disorders including; Parkinsonism and Alzheimer's disease and endocrine disorders including; DM and its complications[16-18]. *C. tamala* is named as patra, tejapatra in Ayurveda books and described properties and actions of this plant. This plant is also used in the therapeutic practice of various diseases or disorders such as cancer, cardiac diseases, diabetes, anxiety, depression, ulcer, GI diseases, and possess many pharmacological activities such as anti-oxidant, anti-hypercholesterolemia, anti-diarrhoeal, anti inflammatory, anti-fungal and antibacterial. According to the research findings, both the stem bark and leaves of *C. zeylanicum* had shown anti-diabetic, *in vitro* anti-amylase, anti-cholinesterase activity, and the ability to manage the complications of T2DM[19]. Therefore, due to the unavailability of *C. tamala* in Sri Lanka, in this study, leaves of *C. zeylanicum* were used as a substitute for *C. tamala* as *Patra*.

Elettaria cardamomum seeds (*Ela*) or small green cardamom, the other ingredient of *Trijata* is known as the "Queen of Spices" belonging to the family Zingiberaceae which is known to have many medicinal properties such as; anti-inflammatory, antiseptic, expectorant, purgative, laxative, carminative, antispasmodic, stimulant, antibacterial, anthelminthic, aphrodisiac, abortifacient, hepatoprotective, cardiotonic, diuretic, antidote to snake venom, anti-ulcerogenic, emmenagogue, cephalic, stomachic and sialagogue properties[20]. *E. cardamomum* in which the studies had shown its effectiveness on glycemic control, lipid profile, and oxidative stress in patients with T2DM.Therefore,due to the fact that both *C. zeylanicum* and *E. cardamomum* would provide a synergistic effect in treating T2DM.

C. tamala, the third ingredient of the formula is named as *Patra* and *Tejapatra* in Ayurveda books with its properties and actions. Compared with *C. zeylanicum,* this plant is also used in similar therapeutic practice of various diseases or disorders such as cancer, cardiac diseases, diabetes, anxiety, depression, ulcer, GI diseases, and possess many pharmacological activities such as anti-oxidant,

anti-hypercholesterolemia, anti-diarrhoeal, anti inflammatory, anti-fungal and antibacterial. Therefore, due to the unavailability of *C. tamala* in Sri Lanka, in this study, leaves of *C. zeylanicum* were used as a substitute for *C. tamala*

Therefore, present study was focused to assess the quality control parameter for the standardization of the new formula of *Trijata* with the stem bark and leaves of *C. zeylanicum* and seeds of *E. cardamomum*[21].

Materials and Methods

Plant material

Mature peeled barks and leaves of *C. zeylanicum* (*Sri Gemunu* variety) were collected in three batches from the Cinnamon Research Station, the National Cinnamon Research Station, Nilambe, Peradeniya (PD/CZ121). *E. cardamomum* seeds (*Vazhukka* variety) were collected in three batches from the Department of Export Agriculture, Kandy Road, Matale. Specimens were authenticated from the National Herbarium, Department of National Botanical Gardens, Peradeniya, Sri Lanka, and voucher specimens were deposited (PD/EC112).

Chemicals

All the chemicals used for the study were of analytical grade.

Preparation of powders of *C. zeylanicum* leaves, barks, and *E. cardamomum* seeds

Fresh plant materials were washed under running tap water to remove dust and dirt, rinsed with distilled water, air-dried, weighed, oven-dried until a constant weight was obtained at a temperature below 40°C, powdered, and sieved with the mesh size of 125 µm (No. 120 sieve).

Formulation and evaluation of NT value-added product

A novel *Trijata* (NT) was formulated incorporating the leaves, barks of *C. zeylanicum,* and seeds of *E. cardamomum* in 1:1:1 proportion. It was subjected to microscopic identification, determination of physico-chemical parameters including; total ash, water-soluble ash, and acid insoluble ash, qualitative tests including; phytochemical screening tests, quantitative tests including total phenolic content (TPC), total flavonoid content (TFC), antioxidant assays, microbiological limits, extractable matter and presence of heavy metals. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) were run for the final product of NT.

Preparation of extracts

Hot Soxhlet extraction was followed with distilled water and methanol. Cold extraction was followed with distilled water and methanol to obtain the respective extracts as per the WHO guidelines[22].

Analysis of physico-chemical parameters

Total ash, water-soluble ash, and acid insoluble ash values were determined for each ingredient and NT formulation separately according to WHO Guidelines[22].

Qualitative tests

Preliminary phytochemical screening

Hot water extract and methanol extract were subjected to preliminary phytochemical screening tests to screen for the phytoconstituents such as; phenols, flavonoids[23].

Quantitative tests

Total phenolic content (TPC)

TPC was determined using the Folin-ciocalteu method. Sample, blank and standard (1.0 mL) were added to 60.0 mL of distilled water in a 100.0 mL volumetric flask. Folin-ciocalteu reagent (5.0 mL) was added and mixed. After 1 min and without exceeding 8 min, 15.0 mL of 20% sodium carbonate solution was added and the volume was adjusted to 100.0 mL. The colour generated in the vial after 2 h was observed at wavelength 760 nm. Distilled water with the reagent was used as the blank. A gallic acid calibration curve was prepared to calculate the TPC and the result was expressed as mg gallic acid equivalents (GAE) to 100 g dry weight (DW) of leaves[24].

Total flavonoid content (TFC)

TFC was determined using the aluminium chloride method. Extract (0.10 mL) was placed in a 10.0 mL volumetric flask and distilled water (4.0 mL) was added. $NaNO_2$ (0.30 mL) was added and mixed well. $AlCl_3$ solution (1:100) was added after 5 min. After 1 min, 2.0 mL of 1 M NaOH solution was added and the final volume was topped up to 10.0 mL with distilled water. Absorbance was measured against a blank at wavelength 510 nm. A standard calibration curve of Quercetin was prepared and the total flavonoid content was expressed as mg Quercetin equivalents (QUE) to 100 g DW[24].

Antioxidant activity

2,2- diphenyl-1-picrylhydrazyl assay

Antioxidant capacity was determined using a 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay according

to the method described in Takatsuka[25]. The absorbance values were read at wavelength 515 nm with reference to methanol. The results were expressed as mmol Trolox equivalents (TE) per 100 g DW.

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{The absorbance of the control}} \times 100$$

2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assay

Antioxidant capacity was determined using 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). Absorbance readings were taken at wavelength 734 nm. A standard calibration curve of Trolox was prepared and the percentage inhibition was calculated.

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{The absorbance of the control}} \times 100$$

Extractable matter

The number of active constituents in the formulation extracted with each solvent under hot Soxhlet extraction and cold extraction methods were determined according to WHO guidelines.

Microbiological limits

Microbial content was determined as per the SLS guidelines.

Aerobic plate count, CFU/ g - SLS 516/1: 2013
Coliforms/ g - SLS 516/3: 2013
E. coli/ g - SLS 516/12: 2013
Staphylococcus aureus, CFU/ g - CML/MM/01/02/004
Yeast & Moulds, CFU/ - SLS 516/2, Sec 2-2013
Salmonella, CFU/ g - SLS 516/5: 2017

Heavy metals

Amounts of heavy metals present in the formulation such as lead, cadmium, arsenic, and mercury were quantified as microwave digestion / ICP-MS.

Thin layer chromatography

Normal phase TLC was conducted for methanol extracts of *C. zeylanicum* leaves, barks, and *E. cardamomum* using TLC silica gel 60 f_{254} plates (precoated sheets ALUGRAM Xtra SIL, 2.0 cm x 10.0 cm x 0.2 mm thickness) (Sigma Aldrich). Reverse phase TLC was run for the hot aqueous extract of NT using reverse phase TLC silica gel 60 f_{254} plates (precoated sheets ALUGRAM Xtra SIL, 2.0 cm x 10.0 cm x 0.2 mm thickness) (Sigma Aldrich). Spots were viewed under UV light wavelength. A solvent system was selected with a better separation[26,27].

High-performance thin layer chromatography

All three ingredients were spotted on one reverse phase TLC plate and kept in the photo documentation chamber of CAMAG REPROSTAR 3 with the solvent system. The TLC plate was scanned at UV light of wavelength, 254 nm. Peak display and peak value table were obtained using the software, WINCATS.

Statistical analysis

Statistical Package for Social Sciences (SPSS) 25 was used to analyze the results. All experimental measurements were carried out in triplicate and results were expressed as the mean ± standard deviation. Descriptive statistics were followed to obtain the mean and standard deviation values. One-way Analysis of Variance (ANOVA) was conducted to determine the significant differences between ash values, TPC, TFC, and antioxidant activity at $p<0.05$ was considered as significant at 95% level of confidence.

Results

Analysis of physico-chemical parameters

Total ash, water-soluble ash and acid insoluble ash values in weight/ weight percentage of each ingredient and NT in which means followed by different letters along a column are significantly different at $p<0.05$ (Table 1). Significantly different values were obtained for total ash, water-soluble ash, and acid insoluble ash values for each ingredient and NT as 4.6±0.2%, 3.1±0.1%, and 0.6±0.2% on a DW basis which can be used to minimize the batch-to-batch variation during the further studies. Antioxidant capacity for IC50 concentration of NT hot aqueous and cold aqueous extracts were 251.15±6.21% and 234.89±5.13% respectively as per DPPH radical scavenging activity. Antioxidant capacity determined by ABTS radical scavenging activity for IC50 concentration of NT hot and cold aqueous extracts was 80.38±3.35% and 59.67±2.86% respectively.

Qualitative tests of phytochemical screening

Phytochemical screening tests showed that except for steroids; many phytochemicals were available in both methanol and hot aqueous extracts of NT. Reducing sugars were absent in methanolic extract

Table 1 — Phytochemical and physicochemical profile of the powders of ingredients and NT

Phytochemicals	CL		CB		ES		NT	
	ME	HWE	ME	HWE	ME	HWE	ME	HWE
Alkaloids (Dragendroff's)	+	+	+	+	+	+	+	+
Alkaloids (Mayer's)	+	+	-	+	+	+	+	+
Flavonoids	+	-	-	-	-	-	+	+
Tannins	+	+	+	+	-	-	+	+
Phenols	+	+	+	+	-	-	+	+
Terpenoids	+	-	+	+	+	-	+	+
Saponins	+	-	+	+	-	+	-	+
Reducing sugars	+	-	-	+	-	-	-	+
Amino acids	+	-	-	-	-	-	-	-
Ash Values (w/w %)	CL		CB		ES		NT	
Total ash	4.7±0.1[a]		4.97±0.1[b]		4.99±0.1[c]		4.6±0.2[d]	
Water soluble ash	2.4±0.1[e]		3.2±0.1[f]		0.6±0.1[g]		3.1±0.1[h]	
Acid insoluble ash	0.9±0.1[i]		0.6±0.1[j]		0.98±0.1[k]		0.6±0.2[l]	

Methanol extract (ME) and hot aqueous extract(HWE) CL: *C. zeylanicum* leaves; CB: *C. zeylanicum* barks; ES: *E. cardamomum* seeds and NT: Novel *Trijaha*. Total ash, water-soluble ash and acid insoluble ash values in weight/ weight percentage of each ingredient and *Trijatha* formulation in which means followed by different letters along a column are significantly different at $p<0.05$.

while in the hot water extract, they could be observed in the bark of *C. zeylanicum*. Similarly, flavonoids were only present in the methanolic extract of *C. zeylanicum* leaves (Table 1). It was found that except steroids, many phytochemicals were available in both methanol and hot water extracts.

Quantitative Tests of Total phenolic Content (TPC) and Flavonoid Contents (TFC)

The hot water extract of NT showed a significantly higher amount of TFC (0.078±0.06) when compared with the cold-water extract (0.020±0.01). There was no significant difference between TPC and TFC values in hot water extract (0.42±0.03) and cold-water extract (0.41±0.23). Values were determined in triplicate and means followed by different letters down a row are significantly different at $p<0.05$. These results revealed that higher amount of phenolic and flavonoid contents presented in hot water extract of NT when compared with the cold-water extracts. Total flavonoid content in hot water extract was considerably higher when compared with cold water extract. In the quantification of the TPC, they were 0.42±0.03 mg GAE/ g and 0.41±0.23 mg GAE/ g on a DW basis for the hot and cold aqueous extracts respectively. TFC values for the hot and cold aqueous extracts of *Trijata* were 0.078±0.06 mg QUE/ g and 0.020±0.01 mg QUE/ g on a DW basis.

***In-vitro* antioxidant activity**

2,2- diphenyl-1-picrylhydrazyl Assay

Data were represented as mean ± standard deviation with the number of extracts; n = 6. Radical scavenging activity of hot and cold aqueous extracts was dose-dependent which increased with increasing concentration. The highest values of radical scavenging percentages were obtained for the IC_{50} concentrations of each extract. The DPPH radical scavenging percentages of IC_{50} concentrations were significantly different in hot and cold aqueous extracts ($p<0.05$). The value was higher in the hot aqueous extract compared to the cold aqueous extract (Table 2).

Table 2 — Percentages of DPPH radical scavenging activity of hot and cold aqueous extracts of NT at different concentrations.

Concentration (µg/ mL)	DPPH Radical Scavenging % of HAE	DPPH Radical Scavenging % of CAE
100	20.85±1.58	22.87±1.12
150	28.28±1.21	34.28±1.17
200	37.41±1.75	43.68±0.64
250	52.53±1.73	54.91±0.66
300	60.31±1.35	63.14±1.25
350	70.17±1.71	71.20±0.44
400	78.48±2.70	80.61±0.44
IC_{50}	251.15±6.21[q]	234.89±5.13[r]

HAE: Hot aqueous extract and CAE: cold aqueous extract. Values are expressed as mean ± SD determined in triplicate which means followed by different letters along a column are significantly different at $p<0.05$ (n=6); a-d Means with different superscript letters in the same row indicate differences at $p<0.05$. Mean within each column followed by the same letter are not significantly different at p=0.05.

ABTS Assay

Data were represented as mean ± standard deviation with the number of extracts; n = 6. It showed a dose-dependent ABTS radical scavenging percentage in hot and cold aqueous extracts which increased with the increasing concentration. The highest difference was

Table 3 — ABTS radical scavenging activity of NT aqueous extracts

Concentration (µg/ mL)	ABTS Radical Scavenging % of HAE	ABTS Radical Scavenging % of CAE
25	17.68±2.35	22.17±2.51
50	32.31±5.47	42.70±2.83
75	47.97±4.27	64.99±1.86
100	63.20±2.21	78.62±3.89
125	74.58±1.82	88.66±1.17
IC_{50}	59.67±2.86^{s}	80.38±3.35^{t}

Percentages of ABTS radical scavenging activity of hot and cold aqueous extracts of NT at different concentrations. Values were determined in triplicate (mean ± SD), significantly different at $p<0.05$.

obtained in 75 µg/ mL and 100 µg/ mL concentrations of each extract. The ABTS radical scavenging percentages of IC_{50} concentrations were significantly different in hot and cold aqueous extracts ($p<0.05$). The value was higher in the hot aqueous extract compared to cold aqueous extract which is similar to the antioxidant activity obtained for each extract in the DPPH assay (Table 3).

Extractable matter

Hot aqueous (13.4±0.0^{x}) and methanol extract (10.3±0.1^{v}) of NT showed higher value of extractable matter (EM) compared to cold aqueous (9.5±0.4^{w}) and methanol extracts (8.6±0.0^{u}). The highest EM values were obtained with water compared to methanol in which the EM values were significantly different between the two extraction methods and the solvents. Values were determined in triplicate of which means followed different letters down a row are significantly different at $p<0.05$.

Microbiological limits of NT

Findings showed that the samples of the prepared NT were safe from microbiological contamination and satisfied the WHO guidelines. Coliforms, *E. coli*, and *Salmonella* were not detected.

Aerobic plate count, *Staphylococcus aureus* and Yeast & Moulds counts also determined following consequentially as CFU/g -1.4 x 10^4, CFU/g< 10 and CFU/g 1.2 x 10^2.

Heavy metals contents of NT

Heavy metal analysis showed that all the heavy metals present in the drug were within the safe limits according to the WHO guidelines as follows Lead (Pb) - 1.6 mg/ kg, Cadmium (Cd)-0.16 mg/ kg, Arsenic (As)-0.16 mg/ kg, Mercury (Hg)-0.19 mg/ kg.

Thin layer chromatography

Solvent systems were determined Toluene: Ethyl acetate: Formic acid: methanol 3: 3: 0.8: 0.2 proportion for the ingredients and NT methanol extract. TLC plate showed 3 spots for *C. zeylanicum* bark aqueous extract while it showed 2 peaks for *C. zeylanicum* leaf aqueous extract. Two spots were shown by *E. cardamomum* seed aqueous extract (Fig. 1).

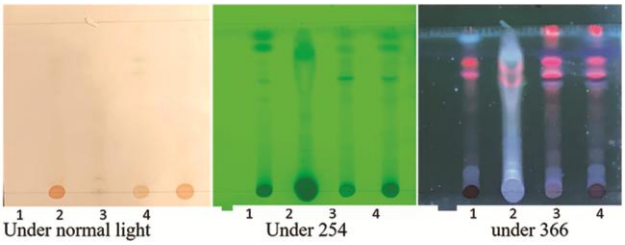

Fig. 1 — TLC of Methanol extract (ME) of *C. zeylanicum* barks (1); *E. cardamomum* seeds (2); *C. zeylanicum* leaves (3); and NT: Novel *Trijaha* (4)

Figure 1: TLC of Methanol extract (ME) of 1:*C. zeylanicum* barks; 2: *E. cardamomum* seeds; 3: *C. zeylanicum* leaves; and 4:NT: Novel *Trijata*.

High-performance thin layer chromatographic analysis of ingredients and NT

HPTLC profile of methanol extract of *C. zeylanicum* bark showed 3 peaks for the solvent system; toluene: ethyl acetate: formic acid: methanol (3: 3: 0.8: 0.2**)** with corresponding range of R_f values; 0.45-0.60, 0.70-0.73, 0.86-0.91. Methanol extract of *E. cardamomum* showed 1 peak the range of R_f value; 0.45 – 0.49. *C. zeylanicum* leaf methanol extract showed 2 peaks with range of R_f values; 0.45-0.49, 0.76-0.83. NT showed 3 peaks with the range of R_f values; 0.47-0.60, 0.73-0.81, 0.82-0.89 (Table 4). HPTLC fingerprints can be used in proper identification and authentication of these raw materials (Fig. 2). It was suggested that the NT formulation would contain synergistic therapeutic effects when compared with its separate ingredients.

Discussion

The present study was focused on a modification of an Ayurveda formula called *Trijata* which contains *C. zeylanicum*, *E. cardamomum*, and *C. tamala*. A modification was introduced since it is difficult to prepare the standard formula of this medicine in the Ayurveda drug manufacturing industry due to the unavailability of *C. tamala* in Sri Lanka. The name "Trijata" was given in classical formula denoting that it contains three ingredients. However, in the present study we used three parts, leaves and bark of C. *zeylanicum* and fruit of the E. *cardamomum* suggesting that reconsideration of the name Trijata.

Table 4 — Peak values of HPTLC analysis of of ingredients and NT

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End position	End height	Area	Area %
1	1	0.45 Rf	7.6 AU	0.54 Rf	57.1 AU	54.75%	0.60 Rf	6.3 AU	2184.7 AU	86.38%
1	2	0.70 Rf	4.7 AU	0.71 Rf	21.7 AU	20.82%	0.73 Rf	5.8 AU	144.5 AU	5.77%
1	3	0.86 Rf	0.7 AU	0.89 Rf	25.5 AU	24.43%	0.91 Rf	0.1 AU	196.7 AU	7.85%
2	1	0.45 Rf	1.5 AU	0.48 Rf	12.6 AU	100.00%	0.49 Rf	9.7 AU	181.0 AU	100.0%
3	1	0.45 Rf	3.7 AU	0.51 Rf	20.9 AU	61.19%	0.57 Rf	0.8 AU	748.5 AU	69.06%
3	2	0.76 Rf	1.7 AU	0.80 Rf	13.3 AU	38.81%	0.83 Rf	4.6 AU	335.3 AU	30.94%
4	1	0.47 Rf	5.8 AU	0.54 Rf	63.2 AU	55.50%	0.60 Rf	0.3 AU	1956.5 AU	62.99%
4	2	0.73 Rf	3.5 AU	0.76 Rf	22.6 AU	19.83%	0.81 Rf	3.5 AU	499.2 AU	16.07%
4	3	0.82 Rf	0.1 AU	0.85 Rf	28.1 AU	24.67%	0.89 Rf	0.1AU	650.5 AU	20.94%

Track 1:*C. zeylanicum* barks; Track 2: *E. cardamomum* seeds ;Track 3: *C. zeylanicum* leaves; and Track 4:NT: Novel *Trijaha*

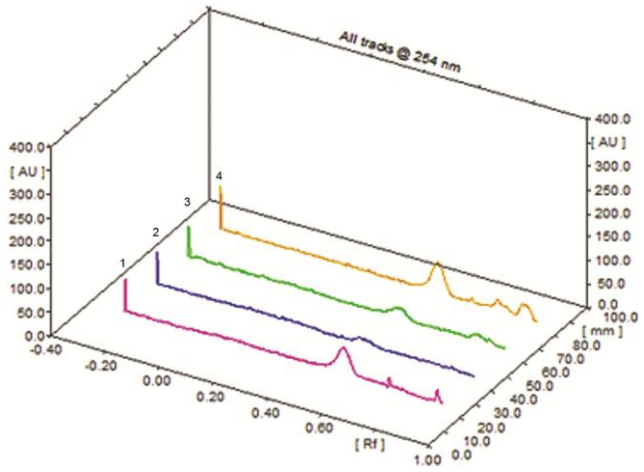

Fig. 2 — display of High-performance thin layer chromatographic analysis of methanol extracts at 254 nm (Track 1: *C. zeylanicum* barks; Track 2: *E. cardamomum* seeds; Track 3: *C. zeylanicum* leaves; and Track 4: NT: Novel *Trijaha*)

Hence, the present study was aimed at developing a new formulation of *Trijata* (NT) using *C. zeylanicum* leaves (*Patra*) as a substitute for *C. tamala*. Further, the modified formula (NT) was subjected to quality control using the drug standardization parameters as the initial step of a new drug formulation. In the present study, ash values are considerably low and are indicating the present formula is less adulterated and contaminated with other undesirable materials or substitutions and also shows the carefulness in the preparation of the drug.

The phytochemical study was applied to identify the main responsible chemical compounds included in the formula. Phytochemical screening and quantifying are one of the most important evaluations in drug standardization and quality control processes. In the present study, the preliminary phytochemical analysis revealed that the phytoconstituents such as; alkaloids, flavonoids, phenols, tannins, and terpenoids are available in both hot and cold aqueous extracts of NT[28-30].

Saponins and reducing sugars were available in the methanol extract of NT. It was suggested that these biologically active compounds are responsible for the therapeutic potential of medicinally important herbs[31-32].

It was further observed that most of the phytochemicals were present in the hot aqueous extract suggesting that when compared with the methanol extract; hot aqueous extract would be more potent in the therapeutic aspects. The hot aqueous extract of NT showed a higher amount of phenolic and flavonoid contents when compared with the cold aqueous extracts further proving that hot aqueous extract would have better therapeutic efficacy. However, it should be further confirmed with clinical trials. Conversely, NT is supposed to have antidiabetic activity and polyphenols of these plant materials would produce this role as several studies have shown that polyphenols contribute to reducing plasma glucose along with elevation of plasma insulin levels

Hence, it could be suggested that the identified constituents would be the responsible chemical constituents for the therapeutic effects.

Despite its antidiabetic capacity flavanones, isoflavones, catechins, etc. have shown protective anticancer effects in a few study models with different pharmacological mechanisms. Moreover, evidence revealed that it is associated with minimizing coronary heart diseases. Therefore, the present study will further investigate their quantitative molecular structural analysis for the confirmation of such activities in NT. Flavonoids are one of the most abundant phytochemicals that have been presented in the current study which are widespread naturally occurring phenolic compounds in the plants. However, it was documented that more than 8000 flavonoids are present in cells. They are accountable for many pharmacological activities which are showing a pivotal role in human health such as antiangiogenic, antiulcer, antiarthritic[32].

The results of TPC and TFC indicated that the NT formulation has high amounts of TP and TF contents which are exhibited through promising antioxidant activity. Further, it showed that the hot aqueous extract has TP and TF contents. This further provides important information regarding the method of drug administration which needs to be considered in future preclinical and clinical studies. Polyphenols are proven for their capability as potent natural antioxidants for preventing oxidative damage which enhances human health[32,33] and high phenolic content in this formula suggest that the NT would possess similar characteristics. Cardamom supplementation boosted antioxidant production and activity in diabetic subjects, reducing oxidative stress and inflammatory markers. It was revealed that the antioxidant capacity is dose-dependent and showed a higher value with hot aqueous extract. It further proves that hot aqueous extract has a considerable pharmacological effect in clinical applications. Evaluation of extractive values is an important process in the assessment of crude drugs. Extractive values reveal the facts of the addition of exhausted material, adulteration, or differences in the preparation in the process of drying, storage, or formulating.

In the present study, the percentage content of active constituents extracted from distilled water by hot Soxhlet extraction and cold extraction was 13.4±0.0% and 9.5±0.4% respectively, while the extractability values are 10.3±0.1% and 8.6±0.0% for hot and cold methanol extracts also studied for further added validation in standardization process. So hot extracts showed higher extractability values which suggests that the hot extraction is more beneficiary in the therapeutic aspect. Contrary, herbal medicinal preparations are commonly used as water extractives or alcoholic extractives in the process of drug manufacturing. These values could be used in further studies as established values for the preparation of the NT. This rationalizes the use of water in the Ayurveda preparation of NT. Microbial contamination and heavy metal analysis are playing an essential role in the drug preparation process. It is assuring the safety of the drug for clinical administration. In the aspect of toxic effects on the systems such as the cardiovascular system, nervous system, reproductive systems, gastrointestinal systems, and major organs such as kidney, liver, and brain, it is necessary to provide the assurance of free heavy metals in the drug. Exposure to heavy metals could lead to severe defects in body systems and organs. In the present study, coliforms, *E. coli*, and *Salmonella* were not detected in microbiological tests. The available species were found within the acceptable microbiological limits. In addition, the heavy metal content was low and within the standard safe limits. Hence, the prepared drug is safe for pre-clinical or clinical evaluation. This will be important in future drug manufacturing and to maintain the batch-to-batch variation. Moreover, these phytochemicals will provide an insight to the pharmacological action of the formula which will be a platform to the future structural analysis of the formula.

Conclusion

The findings of this study are limited to the development of HPTLC profiles. However, these HPTLC profiles would be more helpful to authenticate this product for drug manufacture and further research studies. Results of this study would be used for further studies of quality control and standardization of NT as a value-added herbal product of *C. zeylanicum* and Cardamom which will be beneficial for the Sri Lankan economy. It will enhance the quality of life of *C. zeylanicum* and Cardamom growers of the spice industry and maintain the supply chain of quality raw materials.

Acknowledgments

The authors are thankful to the Ministry of Primary Industry collaborated with the National Science Foundation (NSF), Sri Lanka for the financial support.

Conflict of Interests

The authors declared no conflict of interest.

Author Contributions

SDH: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft and review, Project administration, Supervision. DS: Formal analysis, Investigation, Methodology, Writing - original draft and review

NK: Supervision, Conceptualization, Writing - original draft, review & editing. CM: Formal analysis, Investigation, Writing – review & editing.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

References

1 Singh A, *Bhava Prakasha Nighantu*, English translation, (Chaukambha Publihers, Varanasi), (2007) p. 62

2 Sharma P V & Sharma G P, *Kaiyadeva Nighantu*, Hindi translation, (Chaukambha Orientalia, Varanasi), (1979) p. 250.

3 Jayasinghe D M, Buddhadasa H K, Rajapaksa D H & Jayethilaka K G P, *Ayurveda Pharmacopeia*, (Department of Ayurveda, Colombo, Sri Lanka), 1976.

4 Kumari A & Thewari P, *Yogarathnakara- A complete treatise on ayurveda* (Part one), (Chaukambha Orientalia, Varanasi), 2019.

5 Murthi K R S, *Caraka Samhita by Agnivesha*, English translation, (Chaukambha Orientalia, Varanasi), 2010.

6 Murthi, K R S, *Susruta Samhita*, English translation, 1st ed. (Chaukambha Orientalia, Varanasi), 2010.

7 Murthi, K R S, *Ashthaga Hrgayam by Vagbhata*, English translation, Vol.1, (Krishnadas Acadamy, Varanasi), 1994.

8 Ullah M A & Hassan A, Cinnamon as traditional and modern medicine, *J Agric Hortic Res*, 5 (2) (2022) 141-150.

9 Surendran S & Ramasubbu R, Phytochemistry and pharmacological studies of Indian Cinnamomum Schaeff, In: *Bioprospecting of Tropical Medicinal Plants*, Arunachalam K, Yang X, Puthanpura S, Eds, (Springer, Cham. Publication), (2023) 649-697. https://doi.org/10.1007/978-3-031-28780-0_26

10 Zarezadeh M, Musazadeh V, Foroumandi E, Keramati M, Ostadrahimi A, *et al.* The effect of cinnamon supplementation on glycemic control in patients with type 2 diabetes or with polycystic ovary syndrome: An umbrella meta-analysis on interventional meta-analyses, *Diabetol Metab Syndr*, 15 (2023) 127. https://doi.org/ 10.1186/s13098-023-01057-2

11 Abeysekera W P K M, Premakumara G A S, Ratnasooriya W D & Abeysekera W K S M, Anti-inflammatory, cytotoxicity and antilipidemic properties: novel bioactivities of true cinnamon (*Cinnamomum zeylanicum* Blume) leaf, *BMC Complement Med Ther*, 22 (1) (2022) 259.

12 Mishra N & Srivastava R, Therapeutic and pharmaceutical potential of cinnamon, In *Research Anthology on Recent Advancements in Ethnopharmacology and Nutraceuticals*, (IGI Global Publication), (2022) 698-710.

13 Mohsin S N, Saleem F, Humayun A, Tanweer A & Muddassir A, Prospective nutraceutical effects of Cinnamon derivatives against insulin resistance in Type II Diabetes Mellitus-Evidence from the literature, *Dose-Response*, 21 (3) (2023) 15593258231200527.

14 Wu T, Huang W, He M & Yue R, Effects of cinnamon supplementation on lipid profiles among patients with metabolic syndrome and related disorders: A systematic review and meta-analysis, *Complement Ther Clin Pract*, 49 (2022) 101625.

15 Senevirathne B S, Jayasinghe M A, Pavalakumar D & Siriwardhana C G, Ceylon cinnamon: a versatile ingredient for futuristic diabetes management, *J Future Foods*, 2 (2) (2022) 125-142, ISSN 2772-5669.

16 Yu T, Lu K, Cao X, Xia H, Wang S, *et al*., The effect of cinnamon on glycolipid metabolism: A dose–response meta-analysis of randomized controlled trials, *Nutrients*, 15 (13) (2023) 2983.

17 Ballester P, Cerdá B, Arcusa R, García-Muñoz A M, Marhuenda J, *et al*., Antioxidant activity in extracts from Zingiberaceae family: Cardamom, turmeric, and ginger, *Molecules*, 28 (10) (2023) 4024.

18 Ahmadi A, Naziri M, Fallahpour F, Gholami K, Arabpour J, *et al*., Therapeutic potential of cinnamon for neurological disorders: A mini-review, Neurology Asiam, 27 (1) (2022).

19 Pagliari S, Forcella M, Lonati E, Sacco G, Romaniello F, *et al*., Antioxidant and anti-Inflammatory effect of Cinnamon (*Cinnamomum verum* J. Presl) bark extract after *in vitro* digestion simulation, *Foods*, 12 (3) (2023) 452. https://doi.org/ 10.3390/foods12030452.

20 Nameni G, Moradi Y, Zaroudi M & Jamshidi S, Effect of cardamom supplementation on a number of metabolic factors: A systematic review and meta-analysis, *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 16 (6) (2022) 102523.

21 Farooq F, Hussain Z, Hanief M I & Fida Z, Cinnamon (*Cinnamomum zeylanicum*); a brief review of the culinary spice with its potential therapeutic indications, *Eur J Biomed Pharm Sci*, 10 (6) (2023) 2349-8870.

22 Quality control methods for medicinal plant materials. ISBN 92 4 154510 0 © World Health Organization 1998.

23 Valsan A, Bose A & Kumar A A, Preliminary phytochemical screening of indigenous medicinal plants *Ocimum tenuiflorum*, *Ocimum basilicum* and *Ocimum gratissimum*, *Res J Agric Sci*, 13 (4) (2022) 925-30.

24 Mohammed E A, Abdalla I G, Alfawaz M A, Mohammed M A, Al Maiman S A, *et al*., Effects of extraction solvents on the total phenolic content, total flavonoid content, and antioxidant activity in the aerial part of root vegetables, *Agriculture*, 12 (11) (2022) 1820.

25 Takatsuka M, Goto S, Kobayashi K, Otsuka Y & Shimada Y, Evaluation of pure antioxidative capacity of antioxidants: ESR spectroscopy of stable radicals by DPPH and ABTS assays with singular value decomposition, *Food Biosci*, 48 (2022) 101714.

26 Bunu S J, Okei J O, Miediegha O, Ebeshi B U & Chukwuemerie O L, Assessment of secondary metabolites and thin-layer chromatographic analysis of *Carica papaya* (Caricaceae) leaves ethanolic extract, *J Pharm Res Int*, 35 (36) (2023) 21-28.

27 Khan A D, Singh M K, Lavhale P M & Kaushik R, Phytochemical screening and HPTLC analysis of bio-active markers of ethanol extract of Indian bay leaves, *J Herbs Spices Med Plants*, 29 (2) (2023) 156-67.

28 Mutha R E, Tatiya A U & Surana S J, Flavonoids as natural phenolic compounds and their role in therapeutics: an overview, *Future J Pharm Sci*, 7 (2021) 25. https://doi.org/10.1186/s43094-020-00161-8.

29 Hao B, Yang Z, Liu H, Liu Y & Wang S, Advances in flavonoid research: sources, biological activities, and developmental prospectives, *Curr Issues Mol Biol*, 46 (4) (2024) 2884-2925. doi: 10.3390/cimb46040181. PMID: 38666911; PMCID: PMC11049524.

30 Foss K, Przybyłowicz K E & Sawicki T, Antioxidant activity and profile of phenolic compounds in selected herbal plants, *Plant Foods Hum Nutr*, 77 (3) (2022) 383-389. https://doi.org/10.1007/s11130-022-00989-w.

31 Singhal P K, Gautam G K, Kumar R & Kumar G, A review on *Amomum subulatum* and *Elettaria cardamomum* with their pharmacological activity, *Recent Trends Pharm Sci Res*, 4 (1) (2022) 1-6.

32 Ju J, Santana de Oliveira M, Qiao Y, Adjuvant therapeutic effect of cinnamon on diabetes mellitus, in cinnamon: A medicinal plant and a functional food systems, Cham: Springer International Publishing, 30 (7) (2023) p. 179-196.

33 Srikantha M K R, *Sharanghdhara Samhitha* (Madhyma Khanda) (Chaukamba Orientalia, India), 84-100.