RESEARCH ARTICLE
DOI: https://doi.org/10.4038/ouslj.v20i1.7696

OUSL Journal, 2025
Vol. 20, No. 1, (pp. 5-24)

Phytochemical Profile and Antioxidant Activity of a Value-Added Herbal Tea from *Vernonia cinerea* and *Zingiber officinale* as a Potent Health-Promoting Beverage

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Abstract

The promising therapeutic efficacy of Monarakudumbiya *(Vernonia cinerea)*, a medicinal herb widely used in traditional medicine in Sri Lanka, has significant potential for use as a herbal tea, primarily due to its numerous health benefits. *V. cinerea* exhibits

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(Received 01st March 2025; Revised 20th June 2025; Accepted 30th June 2025)

various pharmacological activities, including antioxidants, anti-inflammatory, and antimicrobial properties, which can be attributed to its rich phytochemical profile, comprising flavonoids, terpenoids, and phenolic compounds. The current study focuses on the development of a value-added herbal tea formulation, which includes *V. cinerea* as the primary ingredient and ginger (*Zingiber officinale*) as a secondary, value-added natural ingredient. The herbal tea blend was optimized during the formulation process to enhance its palatability, chemical composition, and phytochemical content. Phytochemical analysis revealed higher concentrations of phenols, flavonoids, and other bioactive compounds, which contribute to the potent antioxidant and anti-inflammatory properties of the herbal tea blend. The total phenolic content (TPC) was slightly higher in the value-added herbal tea compared to the extract from dried *V. cinerea* leaves; whereas, the total flavonoid content (TFC) showed a significant enhancement in the formulation. However, both TPC and TFC were accompanied by a marked decrease in residue. The herbal tea exhibited a strong free radical scavenging activity, as evidenced by its low IC_{50} value in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, indicating the effective ability to reduce oxidative stress. The addition of *Z. officinale* not only enhanced phenolic content but also introduced complementary bioactive components with anti-inflammatory and anticancer effects. Together, the synergistic properties of *V. cinerea* and *Z. officinale* make this herbal tea a potent health-promoting beverage, promoting immunity and reducing the risk of chronic diseases.

Keywords: *Vernonia cinerea, herbal tea, phytochemicals, chemical composition, antioxidant activity, value-addition*

Introduction

Vernonia cinerea, commonly known as "Monarakudumbiya" in Sri Lanka, is a herbaceous plant from the Asteraceae family. It is widely distributed in tropical and subtropical regions, including South Asia and South America. *V. cinerea* has been used in

traditional medicine for centuries, particularly in Sri Lanka, due to its numerous health benefits, anti-inflammatory and antioxidant properties, which make it effective for conditions such as arthritis, muscle pain, and oxidative stress (Trang et al., 2024). Additionally, *V. cinerea* serves as an antipyretic, antidiabetic, and antimicrobial agent (Alara et al., 2018). *V. cinerea* also promotes digestive health and helps treat issues such as diarrhoea and dysentery. The therapeutic potential of *V. cinerea* is attributed to its rich phytochemical profile, which includes flavonoids known for their antioxidant and anti-inflammatory properties, terpenoids recognized for their antimicrobial and anti-inflammatory effects, phenolic compounds contributing to its antioxidant capacity, alkaloids exhibiting a range of biological activities including antimicrobial effects, and saponins known for their immune-boosting and anti-inflammatory properties (Trang et al., 2024).

Z. officinale, renowned for its anti-inflammatory, antioxidant, and digestive benefits (Gupta et al., 2014), is an ideal candidate for enhancing the value of herbal preparations. Combining *V. cinerea* with *Z. officinale* could enhance the overall health benefits of the resulting herbal tea. This value addition not only improves the taste and palatability of herbal tea but also enhances its nutritional and medicinal properties. Recent studies have scientifically validated many traditional uses of *V. cinerea*, confirming its medicinal properties, including anticancer, anti-inflammatory, analgesic, and antioxidant effects. However, the formulation of herbal tea from *V. cinerea*, its immunity-enhancing properties, and its proximate composition still require scientific validation.

This research study focuses on formulating a herbal tea incorporating *V. cinerea* leaves and *Z. officinale*, aiming to evaluate its potential as an immunity-enhancing functional beverage. This research examines the bioactive components and nutritional profile of the developed herbal tea. It is compared with the dried leaves of *V. cinerea* as the control experiment and the residue left after each tea sample's infusion to assess the value-added and

health benefits derived from its consumption.

The current study involves proximate analysis to determine basic nutritional parameters, such as moisture, ash, fibre, fat, protein, and carbohydrate content, as well as phytochemical analysis to quantify bioactive compounds qualitatively and quantitatively, including carbohydrates, proteins, amino acids, phenols, and other secondary metabolites. Additionally, it measures the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity to evaluate the functional and medicinal properties of the tea. These parameters are essential for understanding the contributions of *Z. officinale* as a value-added component. The development and analysis of the herbal tea blend provides a comprehensive scientific basis for positioning the developed herbal tea as a nutritionally enriched, immunity-enhancing, and health-promoting functional beverage.

Methodology

Apparatus and equipment

The apparatus and equipment used included an electronic analytical balance (Ohaus Corporation, USA), a muffle furnace (Thermo Scientific, USA), a Kjeldahl apparatus (Velp Scientifics, USA), and a UV-visible spectrophotometer (BK-UV 1800 spectrophotometer – BIOBASE, Jinan, China).

Collection and authentication of samples

The whole plant of *V. cinerea* and rhizome of *Z. officinale* were collected from the Colombo District in the Western Province of Sri Lanka and authenticated at the Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Sri Lanka.

Preparation of *V. cinerea* value-added herbal tea

The leaves of *V. cinerea* and rhizomes of *Z. officinale* were air-dried and powdered separately. Then, the powders were sieved through

a sieve of 1 mm to obtain a smooth powder. Three different formulations (Formula F_1, F_2, and F_3) were prepared by mixing powdered samples of *V. cinerea* and *Z. officinale* (exact proportions are not disclosed due to proprietary constraints). The resulting powders were stored in the laboratory at room temperature until they were used. A sample (1.5 g) of each formulation was bagged in rectangular infusion tea bags (5 cm × 4 cm) and stored in an air-tight container at an ambient temperature. Powder of *V. cinerea* was bagged separately as a control.

Preparation of tea

Each tea bag was dipped in 100 mL of hot, boiling water for 2-5 minutes, without added sugar, in a cup. The sample infusions were approximately 60°C to 70°C at the time of tasting. Four different tea samples from each tea formulation (F_1, F_2, and F_3) and the control were prepared.

Sensory evolution

V. cinerea tea samples were scored by a 10-member semi-trained panel. Sample infusions were randomly given to panelists. The herbal tea was served, and panelists were instructed to score on a 5-point Hedonic scale, where 1 = very poor, 2 = poor, 3 = average, 4 = good, and 5 = excellent. The attributes for scoring included colour and appearance, aroma, taste, texture, and overall acceptability.

Chemical analysis of herbal tea

The moisture, ash, fat, and protein content of tea samples were determined according to the Association of Official Analytical Chemists (AOAC, 1990) methods. The crude fibre content and carbohydrate content were determined by the Weende method and the “By difference” method, respectively.

Phytochemical screening

Alkaloids, carbohydrates, and glycosides were identified using Mayer's test, Fehling's test, and Liebermann's test, respectively. Saponins were detected using the foam test. Proteins and amino acids were identified using Biuret's test and the Ninhydrin test, respectively. Phenols were determined by the Ferric Chloride test, terpenoids with the Salkowski test, and steroids via the Libermann test. Flavonoids were detected by the Alkaline reagent test. Aqueous extracts of *V. cinerea* dried powder and its value-added product were used for phytochemical analysis (Balamurugan et al., 2019).

Determination of total phenolic content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu reagent method. 0.1 mL of *V. cinerea* dried leaves, herbal tea extract, and residue of herbal tea extract were mixed with 0.5 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent. After 6 minutes, 1 mL of 7% sodium carbonate and 0.5 mL of distilled water were added to the reaction mixture. The absorbance was measured after 90 minutes using a UV-Visible spectrophotometer at 760 nm. TPC was determined by the calibration curve. Gallic acid was used as the standard reference material. The calibration curve was plotted using gallic acid (0.1 mg/mL to 5 mg/mL) as the standard, and the TPC was expressed as milligrams of gallic acid equivalents per gram of the dried sample (mg GAE/g). All the experiments were performed in triplicate. The results were expressed as mg gallic acid equivalent (mg GAE)/g dried weight.

Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) of each *V. cinerea* herbal tea extract was determined by the aluminium chloride complex forming assay. Quercetin was used as the reference standard to make a calibration curve. The varying concentrations, ranging from 0.1 mg/mL to 5 mg/mL of quercetin were prepared in

methanol. Approximately 0.1 mL of each quercetin dilution was mixed with 0.5 mL of distilled water and then with 0.1 mL of 5% sodium nitrate and allowed to stand for 6 minutes. Then, 0.15 mL of a 10% aluminium chloride solution was added and allowed to stand for 5 minutes. Afterwards, 0.2 mL of a 1 M Sodium hydroxide solution was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on a UV spectrophotometer. The same procedure was repeated with the *V. cinerea* herbal tea extract. The results were expressed as mean values ± standard deviations in mg quercetin equivalents (QUE)/g dried weight (Hossain et al., 2011).

Determination of antioxidant activity

DPPH radical scavenging activity of the extract of herbal tea, control, and residue was determined according to the procedure described by Blois (1958) and Loizzo et al. (2010). To perform the assay, a 0.20 mM DPPH solution (0.0078 g in 100 mL of methanol), extracts of herbal tea (0.25–3 mg/mL), and Trolox (0.025-1 mg/mL) solutions were prepared. An aliquot of 1 mL of 0.20 mM DPPH and 2 mL of the extract from each concentration of herbal tea were mixed. The mixture was shaken vigorously under dim light and then kept in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm. The experiment was carried out in triplicate for each test solution. Methanolic DPPH solution was used as the control. Trolox was used as a positive standard.
The percentage inhibition of the test samples and positive control was calculated by using the following equation.

$$\text{Scavenging activity} = \frac{(A_0) - (A_1)}{(A_0)} \times 100$$

A_0 – absorbance of the control
A_1 – absorbance in the presence of extract

The results are expressed as mean ± standard deviation (SD).

Results and Discussion

Sensory evolution: The panelist reported that the taste and aroma are less in formula F_1 compared to the other two formulas and the control. It was noted that the aroma and taste of formula F_3 have considerably less overall acceptability. The panelists noted that formula F_2 is the best in terms of colour, appearance, aroma, taste, texture, and overall acceptability. Therefore, F_2 was selected as the best formula because it was the most preferred product in terms of colour, flavour, aroma, and overall acceptability.

Proximate analysis

V. cinerea dried leaves, value-added herbal tea, and the residue of herbal tea after drinking were subjected to proximate analysis using the Official Methods of Analysis (AOAC, 1990), as depicted in Table 2, for moisture, ash, crude protein, fat, and carbohydrates. All analyses were carried out in triplicate.

The results revealed a notable increase in ash content and fibre content in the value-added herbal tea compared to the dried *V. cinerea* leaves. Additionally, the fat and protein levels were higher in the value-added herbal tea. Ash content represents the total mineral content of a substance. The high ash content in herbal tea reflects its mineral richness, including potassium, calcium, magnesium, and trace elements, which are essential for immune support, antioxidant activity, and enzymatic functions. These minerals aid in neutralizing reactive oxygen species (ROS), thereby mitigating oxidative stress and reducing the risk of associated diseases (McDowell et al., 2007). The dietary fibre content in herbal tea contributes to improved gut health by supporting gut microbiota, reducing inflammation, and facilitating the removal of toxins (Kumar et al., 2023). Moisture content is an essential determinant of product shelf life, with lower moisture levels correlating to extended stability. The analysis demonstrated that the value-added herbal tea had a reduced moisture content compared to dried *V. cinerea* leaves, suggesting a longer shelf life.

The analysis demonstrated that the value-added herbal tea had a slight increase in the fat and protein content of the herbal tea formula F_2.

The residue obtained after infusion exhibited reduced levels of all measured parameters except for moisture, indicating that the nutritional components, including minerals and dietary fibres, were effectively extracted and consumed. The increased moisture content in the residue is attributed to the addition of water during the tea preparation process. Furthermore, the analysis suggests that incorporating *Z. officinale* into *V. cinerea* powder slightly enhances the nutritional profile of the herbal tea, thereby improving its mineral and dietary value. The findings confirm that these nutrients are bioavailable and consumed by the users.

Table 1. *Results of proximate analysis of V. cinerea dry leaves, formulated herbal tea F_2, and residue of herbal tea after drinking.*

Proximate Parameter	*V. cinerea* dried leaves	*V. cinerea* value-added herbal tea (F_2)	Residue of value-added herbal tea after drinking
Moisture Content	17.09 ± 0.38	15.47 ± 0.85	35.58 ± 0.13
Ash Content	18.71 ± 0.38	26.52 ± 0.90	24.15 ± 0.48
Fat Content	12.02 ± 0.94	13.54 ± 0.12	9.23 ± 0.10
Protein Content	12.11 ± 0.84	13.88 ± 0.16	10.20 ± 0.25
Fiber	15.02 ± 0.14	17.93 ± 0.32	11.17 ± 0.53
Carbohydrate Content	25.03	12.64	11.65

The results are expressed as mean ± standard deviation (SD).

Phytochemical screening

Phytochemical screening was conducted on the aqueous extracts of dried *V. cinerea* leaves, value-added herbal tea, and the residual material remaining after consumption. The results, summarized in Table 2, confirm the presence of various phytochemical components in all three extracts. The value-added herbal tea demonstrated notable increases in carbohydrates, proteins, amino acids, phenols, and tannins compared to the dried *V. cinerea* leaves. Previous studies have identified sesquiterpene lactones as primary secondary metabolites in *V. cinerea*, alongside other terpenoids, phytosterols, flavonoids, quinic acid derivatives, alkaloids, and phenolic compounds (Trang et al., 2024). The observed increase in specific phytochemical components in herbal tea is attributed to the addition of *Z. officinale*, which is known to contain over 60% carbohydrates, 9% proteins, as well as significant quantities of monoterpenes, sesquiterpenes, and phenolic compounds (Kiyama, 2020).

The phytochemical analysis of the herbal tea residue revealed a substantial decrease in phenols and a slight reduction in carbohydrates, proteins, amino acids, and terpenoids compared to the value-added herbal tea, suggesting that these bioactive compounds were effectively extracted into the tea and subsequently consumed.

The identified phytochemical constituents, particularly polyphenolic compounds such as phenols and flavonoids, are recognized for their potent antioxidant properties. These compounds exhibit a range of biological activities, including antimicrobial, antidiarrheal, antiallergic, and antiviral effects (Oluwaseun et al., 2018). Furthermore, they play a role in regulating gene transcription, enhancing gap junction communication, and improving immune function (Kiyama, 2020).

Table 2. *Results of qualitative phytochemical analysis of aqueous extracts*

Phytochemical	*V. Cinerea* dried leaves powder (Control)	Formulated herbal tea from *V. cinerea*	Residue of formulated herbal tea from *V. cinerea* after drinking
Alkaloid	+++	++	++
Carbohydrates	++	+++	++
Glycoside	+	+	+
Saponins	+++	+++	+++
Proteins	+	++	+
Amino acids	++	+++	++
Phenols	++	+++	+
Tannins	+	++	++
Terpenoids	+++	+++	++
Steroids	+	+	+
Flavonoids	+	+	+

+++ Present in considerable amount (positive within 5 minutes)
++ Present in moderate amount (positive within 5-10 minutes)
+ Present in trace amount (positive within 10-15 minutes)
- Completely absent

The enhanced phytochemical composition of the value-added herbal tea, particularly due to the inclusion of *Z. officinale*, underscores its improved nutritional and medicinal properties. The observed reduction of these compounds in the residue further supports their bioavailability and successful consumption. Phenolic compounds, including flavonoids, are considered critical secondary metabolites due to their antioxidant, anti-inflammatory, antimutagenic, and anticancer properties. Thus, determining the

total phenolic and flavonoid content of the value-added herbal tea is crucial to evaluating its potential as an immunity-enhancing herbal tea.

Determination of total phenolic content

The total phenolic content (TPC) of the aqueous extracts of dried *V. cinerea* leaves, value-added herbal tea and the residue of the herbal tea was determined by the Folin-Ciocalteu method, and results are depicted in Table 3.

Table 3. *Total phenolic content present in aqueous extracts*

Aqueous extracts	Total phenolic content (mg/g of the extract as GAE)
V. Cinerea dry leaves powder (Control)	43.000 ± 0.006
Formulated herbal tea	44.167 ± 0.006
Residue of formulated herbal tea after drinking	15.550 ± 0.006

The results are expressed as mean ± standard deviation (SD).

The analysis demonstrated that the TPC was slightly higher in the value-added herbal tea compared to the extract from dried *V. cinerea* leaves. Notably, the TPC in the residue of the herbal tea showed a marked reduction, indicating substantial consumption of phenolic compounds by participants during tea consumption. The incorporation of *Z. officinale* into the herbal tea contributed to the enhancement of phenolic compound content, thereby amplifying its health benefits. Literature reported that *Z. officinale*-derived phenolic compounds exhibit diverse biological activities, including the induction of apoptosis via death receptor mediators in glioblastoma cells, p53-mediated apoptosis in skin tumour cells, and reactive oxygen species (ROS)-mediated apoptosis in colorectal

carcinoma cells (Siles et al., 2015). Additionally, these compounds have been shown to initiate DNA damage responses such as cell cycle arrest and apoptosis, demonstrating efficacy against triple-negative breast cancer cells, myeloid leukemia cells, and cervical cancer cells. Furthermore, *Z. officinale* has been shown to mitigate chemotherapy-induced nausea and vomiting, as supported by a meta-analysis of 10 studies (Kiyama, 2020). These findings highlight the value-added herbal tea's potential health benefits, not only for healthy individuals but also as a supportive intervention to enhance immunity in cancer patients.

Determination of total flavonoids content (TFC)

Flavonoids are a significant class of antioxidants found in medicinal plants, recognized for their ability to neutralize free radicals and their associated biological activities (Abeysinghe et al., 2021). The total flavonoid content (TFC) of aqueous extracts from dried *V. cinerea* leaves, value-added herbal tea, and the residue post-consumption was measured using the aluminum chloride complex formation assay. The results are presented in Table 4.

Table 4. *Total flavonoid content present in aqueous extracts*

Aqueous extracts	Total flavonoid content (mg/g of the extract as QUE)
V. Cinerea dry leaves powder (Control)	21.516 ± 0.005
Formulated herbal tea	32.467 ± 0.005
Residue of formulated herbal tea after drinking	14.216 ± 0.005

The results are expressed as mean ± Standard Deviation (SD).

The analysis revealed a substantial increase in TFC, in the value-added herbal tea compared to the dried *V. cinerea* leaves extract. Additionally, a significant reduction of TFC was observed in the

residue compared to the value-added herbal tea, indicating that a considerable amount of flavonoids were consumed during tea intake. Flavonoids are known for their multifunctional antioxidant properties, including metal ion chelation, free radical scavenging, and activation of antioxidant enzymes. These mechanisms contribute to the prevention or mitigation of oxidative stress-related conditions such as diabetes, cancer, cardiovascular diseases, and renal disorders (Goyal et al., 2017). The results suggest that the value-added herbal tea offers enhanced health benefits, making it a valuable dietary source of flavonoids that promote overall health and reduce the risk of diseases associated with free radicals.

Determination of antioxidant activity

The antioxidant potential of *V. cinerea* dried leaves, value-added herbal tea, and the residue post-consumption was evaluated using the DPPH radical scavenging assay. This assay employs a stable free radical, DPPH, to measure the radical scavenging activity of the extracts. Antioxidants in the extracts reduce the absorbance of DPPH, changing its characteristic purple colour to yellow, indicative of radical neutralization. The IC_{50} value, representing the concentration required to inhibit 50% of the radicals, was used to compare antioxidant efficacy. A lower IC_{50} value corresponded to higher radical scavenging activity. Trolox was used as the positive control. The IC_{50} value for *V. cinerea* dried leaves was 1.92 ± 0.05 mg/mL, indicating substantial antioxidant activity, primarily attributed to its phenolic compounds and flavonoids. Similarly, the value-added herbal tea exhibited an IC_{50} value of 1.98 ± 0.02 mg/mL, as shown in Table 5, demonstrating an antioxidant capacity nearly identical to that of the dried leaves. These results suggest that the addition of *Z. officinale* to the herbal tea does not significantly alter its antioxidant potential, potentially due to complementary or masking effects between the bioactive components of *Z. officinale* and *V. cinerea*.

Table 5. *IC50 values of aqueous extracts and Trolox standard*

Samples	[IC_{50}]value (ppm)
Trolox	0.19 ± 0.02
V. Cinerea dry leaves powder (Control)	1.92 ± 0.05
Formulated herbal tea	1.98 ± 0.02
Residue of formulated herbal tea after drinking	2.29 ± 0.02

The results are expressed as mean ± standard deviation (SD).

The IC_{50} value for the residue of the infused herbal tea was slightly higher, at 2.29 ± 0.02 mg/mL, indicating a reduction in antioxidant activity. This result suggests that most of the active compounds are water-soluble and are effectively extracted into the tea during infusion, resulting in a residue with diminished radical scavenging properties. The IC50 value was calculated using the concentration versus % inhibition of the DPPH radical curve, as shown in Figure 1A. The antioxidant potential of *V. cinerea* dried leaves, herbal tea, and residue after drinking was compared with that of Trolox, and the concentration vs % inhibition curve is depicted in Figure 1B. These findings highlight that both the dried leaves and the value-added herbal tea effectively scavenge DPPH radicals, providing substantial antioxidant activity. The formulated tea, enriched with *Z. officinale*, offers significant health benefits to consumers by enhancing antioxidant defense and supporting immune function.

The value-added herbal tea, formulated with *V. cinerea* leaves and *Z. officinale*, offers significant health benefits, particularly for immunity enhancement. The tea formula demonstrates superior nutritional value, with increased ash, fibre, protein, and carbohydrate content compared to dried leaves, reflecting its enriched dietary profile.

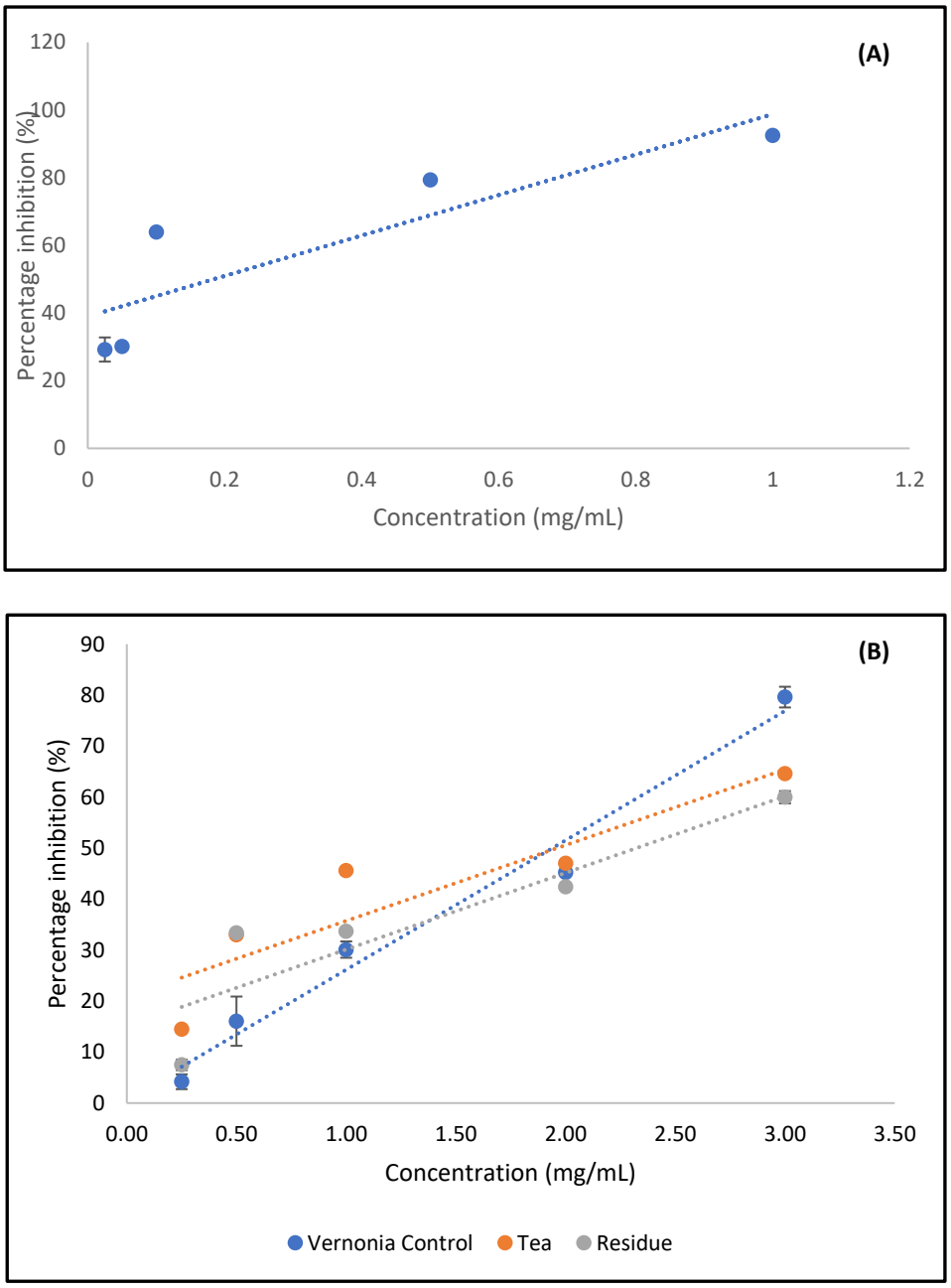

Figure 1. DPPH scavenging assay of Trolox (1A), Dried *V. cinerea* leaves, Value added herbal tea and residue of herbal tea (1B)

Phytochemical analysis revealed higher concentrations of phenols, flavonoids, and other bioactive compounds, which contribute to its potent antioxidant and anti-inflammatory properties. The tea exhibited strong free radical scavenging activity, as evidenced by its low IC_{50} value in the DPPH assay, indicating its effective ability to reduce oxidative stress. The successful extraction and consumption of these bioactive compounds were confirmed by their reduction in the residue, ensuring optimal health benefits for consumers. The addition of *Z. officinale* not only enhanced phenolic content but also introduced complementary bioactive components with anti-inflammatory and anticancer effects. Together, the synergistic properties of *V. cinerea* and *Z. officinale* make this herbal tea a potent functional beverage, promoting immunity and reducing the risk of chronic diseases.

Conclusions

The above study focused on the development of a value-added herbal tea formulation that includes *V. cinerea* as a primary ingredient, with *Z. officinale* serving as a secondary, value-added natural ingredient. The blend was optimized during the formulation process to enhance its palatability, chemical composition, and phytochemical content. This tea demonstrates superior nutritional value, with increased ash, fat, fibre and protein content compared to dried leaves, reflecting its enriched dietary profile. Phytochemical analysis revealed higher concentrations of phenols, flavonoids, and other bioactive compounds, which contribute to their potent antioxidant and anti-inflammatory properties. The combined synergistic effects of *V. cinerea* and *Z. officinale* make this herbal tea a powerful functional beverage that supports immune health and helps lower the risk of chronic diseases.

Acknowledgements

The authors would like to acknowledge the AHEAD-funded research grants provided through the Industry Liaison Centre (ILC)

of the Open University of Sri Lanka.

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