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Optimizations of antioxidants-rich herbal tea formulation from selected medicinal plants for the enhancement of *Psidium guajava* tea

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***Abstract*—Herbal teas are simple, effective, inexpensive, drug-free, and caffeine-free ways to enjoy the flavor and benefits of herbs and spices, which are commonly consumed by people since they are natural, harmless, and can assist in treating or controlling various therapeutic illnesses. The purpose of this study was to develop guava leaf-based herbal tea with a combination of supporting and activating herbs such as *Cinnamomum zeylanicum* (cinnamon) leaf and bark, *Senna auriculata* (avaram senna) flower, *Gymnema sylvestre* (gurmar), *Ocimum tenuiflorum*(holy basil) leaf, and *Citrus limon* (lemon) leaf in order to improve guava tea. Herbal teas were prepared using nine various combinations of the above-mentioned plant materials along with guava tea for comparative purposes. The extraction of tea was done by infusing tea bags, and phytochemicals were screened with a standard procedure. Total polyphenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin-Ciocalteu approach and the aluminum chloride spectrophotometric method, respectively. Total antioxidant capacity was determined by the ferric reducing antioxidant power (FRAP) assay, while radical scavenging activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The toxicity of developed tea bags was assessed using a brine shrimp micro-well cytotoxicity assay. According to the findings, all of the prepared herbal teas and guava tea are rich in essential phytochemicals. Moreover, the combination of guava leaf, cinnamon bark, cinnamon leaf, avaram senna flower, gurmar leaf, holy basil leaf, and lemon leaf (35:20:1:1:1:1:1) exhibited the highest TPC (2027.12 mg GAE/ml) and antioxidant capacity (82.33 mg ascorbic acid eq/ml). During sensory evaluation, this composition was most preferred to guava tea by the semi-trained panel, and it showed nontoxicity to brine shrimp (LC50 values higher than 1000 mg/L). In conclusion, even though guava tea has already been developed and proven to have anti-diabetic and antioxidant properties, the herbal tea formulation from seven medicinal plants showed the greatest preference due to the highest TPC and highest antioxidant capacity. Further studies are needed to determine the anti-diabetic properties of developed herbal teas and compare the anti-diabetic properties of guava tea.**

***Keywords*—**Antioxidants, Guava-tea, Herbal-tea, Phytochemicals, Polyphenolics

I. Introduction

Tea is the most popular beverage in the world, but its origins can be traced back to China, which is gaining public attention as a result of rising awareness of the health advantages resulting from its consumption.(Kochman *et al*., 2020). It is produced by harvesting, processing, and packing leaves from the *Camellia sinensis* plant, which has evolved into nutraceuticals and functional food products (Dufresne Farnworth, 2001). Nowadays, the consumption of herbal tea is widespread and firmly ingrained among our population because allopathic medications are so expensive (Ravikumar, 2014; Toda, 2011). People are increasingly turning to herbal therapies to cure a variety of illnesses due to their rich source of polyphenolic flavonoids, which have strong antioxidant properties, despite the fact that there hasn't been much research on the production of flavored green tea products (Namdev Gupta, 2015). Herbs and herbal polyphenols play a role in regulating oxidation and lowering oxidative damage (Kokilananthan *et al*., 2020). It has been demonstrated that herb teas with high total polyphenol content have strong antioxidant properties, which directly support anti-diabetic properties (Bulugahapitiya *et al*., 2021). An antioxidant is a substrate that helps to manage oxidation, prevents oxidation-induced damage, and reduces oxidative stress in cells (Krishnaiah *et al*., 2011). As a result, it helps treat a variety of human ailments, including diabetes, cancer, cardiovascular disease, and inflammatory disorders, by scavenging free radicals and other unstable chemicals that harm cells in the body (Alkadi, 2020; Sarian *et al*., 2017). Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind(Krishnaiah *et al*., 2011). Some researchers suggest that two-thirds of the world's plant species have medicinal value. Approximately

50% of medicines are made of natural basic materials in the market(Maritim *et al*., 2003; Oberley, 1988). Interestingly, many of the active ingredients in medicinal plants cannot yet be prepared synthetically therefore, the market highly demand for medicinal herbs(Nille Reddy, 2015). In particular, many medicinal plants have great antioxidant potential(Liyanagamage *et al*., 2020). Since many plants have been used for the treatment of diabetes, such as *Psidium guajava*, *Cinnamomum zeylanicum*, *Senna auriculata* Aloe vera, *Gymnema sylvestre*, *Ocimum tenuiflorum*, *Allium sativum, Annona squamosa, Catharanthus roseus, Coccinia grandis, Asparagus racemosus, Bougainvillea spectabilis*, and *Citrus limon*, they help lower the blood glucose levels of the diabetic patient(Luo *et al*., 2019; Musa *et al*., 2011).

Therefore, the study was aimed at developing guava tea by incorporating some other medicinal plants, including *Cinnamomum zeylanicum* (cinnamon) leaf and bark, *Senna auriculata* (avaram senna) flower, *Gymnema sylvestre* (gurmar), *Ocimum tenuiflorum*(holy basil) leaf, and *Citrus limon* (lemon) leaf, in order to improve guava tea by increasing the polyphenolic content in herbal tea.

II. Materials and Methods

A. Plant collection

Fresh *Psidium guajava* (guava) leaf, *Senna auriculata* (avaram senna) flower, *Gymnema sylvestre* (gurmar), *Ocimum tenuiflorum*(holy basil) leaf, and *Citrus limon* (lemon) leaf were taken from a household garden in Batticaloa (latitude 7.7060 ºN, longitude 81.6998 ºE) and fresh *Cinnamomum zeylanicum* (cinnamon) leaf and bark were collected from a household garden in Matara, Sri Lanka (latitude 5.9478 ºN, longitude 80.5483 ºE). The identification of these plants was done at the Department of Biological Sciesnces, Southeastern University of Sri Lanka.

B. Tea preparation

Healthy plant materials were washed with running water multiple times after plant collection and air-dried for two weeks. To be employed in the preparation of tea bags, dried plant leaves were crushed using the grinder. The powdered leaves (1mm) were employed for the preparation of herbal tea. Nine different combinations of herbal teas were prepared by changing the ratio of the above-mentioned plant materials, and guava tea was also prepared for comparison purposes with the prepared herbal tea.

C. Extractions

Prepared tea powder (3.00 g) was used for the extraction by infusion technique for 3 minutes at 98 ºC where 150.0 mL of distilled water was used as a solvent. The extraction process was tripled, and the extracts were used for further chemical analysis.

D. Phytochemical qualitative analysis

Qualitative tests for bioactive compounds such as flavonoids, polyphenolics, alkaloids, saponins, tannins, glycosides, and terpenoids were performed in triplicate for each herbal tea combination and guava tea using the standard qualitative procedures described in the literature(Kokilananthan *et al*., 2022a), (Ekwueme *et al*., 2015), (Shaikh Patil, 2020),((Sawant Godghate, 2013), (Savithramma *et al*., 2011),(Banu Cathrine, 2015) . Tests for Alkaloids: A quantity of 3.0 mL of herbal tea was placed in a test tube, and 1% HCl (1.0 mL) was added. The mixture was then gently boiled for 20 minutes, cooled, and filtered; the filtrates were then tested for the presence of alkaloids using Wagner's reagent and Dragendroff's reagent. Brown or reddish precipitation and creamy precipitation indicate the presence of alkaloids, respectively. Test for Flavonoids (Shinoda Test): Extracts were mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added dropwise. Flavonoids are indicated by color, which is orange, red, pink, or purple, and other assays, like the NH_4OH test and the alkaline reagent test, were also carried out to establish the presence of flavonoids. Test for Polyphenolics/Tannins: The extract was mixed with 2.0 mL of a 2% $FeCl_3$ solution; the presence of polyphenolics/tannins was detected by a blue-green or black color, and a lead acetate test was also carried out for the confirmation of polyphenolics. Test for Terpenoids (Salkowski's Tests): To the extracts, 2.0 mL of chloroform was added. Following that, 2.0 mL of concentrated sulfuric acid was carefully added, softly shaken, and given some time to stand. The interphase was colored reddish-brown to show that terpenoids were present. Similar to this, terpene conformation has also been determined using the Liebermann-Burchardt test. Test for Saponins: Extracts were poured into a test tube and shaken erratically to produce a stable foam, which was used to confirm the saponins' properties. Test for Glycoside (Keller-kilani Test): In a test tube, the extract was mixed with glacial acetic acid (2.0 mL) containing 2 drops of 2% $FeCl_3$, followed by 2.0 mL of concentrated H_2SO_4; a brown ring at the interphase revealed the presence of glycosides.

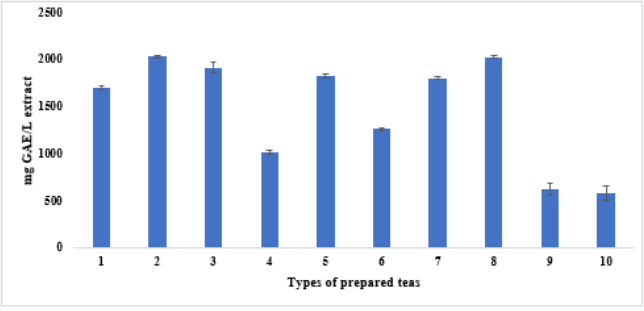

Figure 1: Comparison of TPC of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations).

E. Phytochemical quantitative analysis

Total Phenolic content (TPC): TPC was calculated using a modified Folin-Ciocalteu (FC) method (Prabhavathi *et al*., 2016). A quantity (0.5 mL) of prepared sample extract was added to 2.5 mL of FC reagent combination, and the mixture was allowed to stand for 5 minutes. It was then incubated for 30 minutes with 2.0 mL of a 7.5% w/v Na_2CO_3 solution. The absorbance was measured at 765 nm. Using a gallic acid standard curve, TPC in aqueous extracts was determined and expressed in milligrams of gallic acid equivalents. (mg GAE/g extract)(Kokilananthan *et al*., 2022b), (Ekwueme *et al*., 2015).

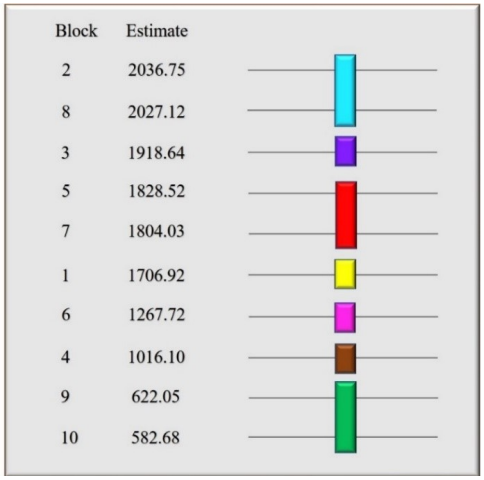

Figure 2: Statistical analysis data of TPC of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations)

Total Flavonoid content (TFC): Utilizing a spectrophotometric technique described in (M. Fernandes, *et al* 2014). In brief, 1.0 ml of the prepared extracts were treated with 0.5 ml of a 2% $AlCl_3$ solution and 0.5 ml of distilled water, and then the absorbance at 425 nm was measured after 10 minutes. Using a quercetin standard curve, the TFC of aqueous extracts was determined and expressed in mg QE/g extract. (Kokilananthan *et al*., 2021), (Pękal Pyrzynska, 2014)

F. Antioxidant analysis

The standard approach outlined in the literature was utilized to determine the radical scavenging activity and total antioxidant capacity of prepared teas using the DPPH radical scavenging assay (DPPH assay) and ferric reducing antioxidant power (FRAP) assay with the help of a UV spectrophotometer. (Shanthirasekaram *et al*., 2021), (Brand-Williams *et al*., 1995)

G. Statistical Analysis

One-way analysis of variance (ANOVA) and a T-test (LSD) are used to analyze and compare the data (LSD: least significant difference). Using SAS, the statistical analysis was carried out. Means and standard deviations were used to report the data.

H. Toxicity assay on brine shrimp

Each nauplii underwent a toxicity assay using a standard protocol reported in the literature in order to determine the concentration (LC_{50}) that would kill 50% of the brine shrimp within 24 hours of exposure (Haniffa, 2021).

I. Determination of physical properties

Selected teas from antioxidant analysis and toxicity assays were subjected to sensory evaluation, which was analyzed by a ranking test with the help of a semi-trained panel (30 panelists), as described in the literature. (Civille Carr, 2015).

J. Moisture contents

In order to calculate the moisture contents of prepared teas (guava, herbal tea 1, 2, and 7), they were subjected to the oven drying method, which is described in the literature (Park Bell, 2004).

III. Results and Discussion

In this study, the infusion method is used to extract herbal powder because infusion is an extraction method that is still usually used to extract phytochemicals. The solvent used in this study is still water because a traditionally used phytomedicine for pharmacological testing is usually used as a water decoction or infusion.

A. Phytochemical qualitative analysis

As per the results of this present study, it showed that all of the herbal tea blends and guava tea contained highly significant secondary metabolites, such as polyphenolics, flavonoids, tannins, terpenoids, and glycosides which is shown in Table I. All of the herbal tea blends and guava tea were discovered to be devoid of alkaloids.

B. Phytochemical quantitative analysis

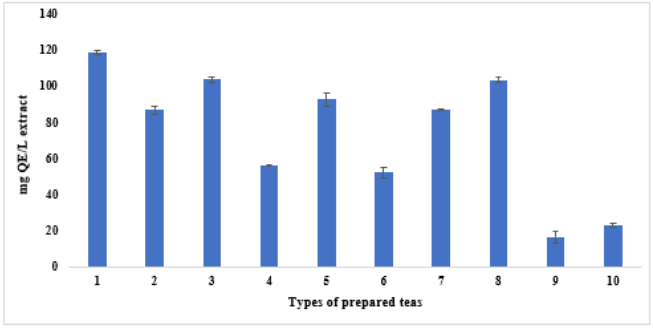

Figure 3: Comparison of TFC of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations).

Researchers have been involved in searching for the medicinal properties of plants that consist of active constituents as well as their antioxidant properties(Gopalakrishnan *et al*., 2016) since humans are becoming resistant to many existing oxidants. As per the results of this present study, the TPC of five prepared combinations of herbal tea was higher than that of guava tea (1706.92 mg GAE/L), which is shown in Figure

Table I: Phytochemical constituents of herbal and guava tea

Phytochemicals	Test	Guava Tea	Herbal Tea 1	Herbal Tea 2	Herbal Tea 3	Herbal Tea 4	Herbal Tea 5	Herbal Tea 6	Herbal Tea 7	Herbal Tea 8	Herbal Tea 9
Flavonoid	Alkaline test	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Shinoda test	+++	++	++	++	++	++	++	++	+	+
	Ammonia test	+++	++	++	+++	++	+++	++	++	+	+
Alkaloids	Wagner's test	-	-	-	-	-	-	-	-	-	-
	Dragendroff's test	-	-	-	-	-	-	-	-	-	-
Phenols	Ferric chloride test	+++	+++	+++	+++	+++	+++	+++	+++	+	+
	Lead acetate test	+++	+++	+++	+++	+++	+++	+++	+++	+	+
Tannins	Braymer's test	+++	+++	+++	+++	+++	+++	+++	+++	+	+
Terpenoid	Salkowski's test	+++	++	++	+	++	+	++	+++	+	+
	Liebermann-Burchardt test	+++	++	++	+++	++	+++	++	+++	++	+
Glycoside	Keller- Kilani test	+++	+++	++	+	++	+	++	+++	+	+
Saponins	Froth test	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

(+++) strong positive reaction, (++) moderate positive reaction, (+) Weak positive reaction, (-) no reaction.

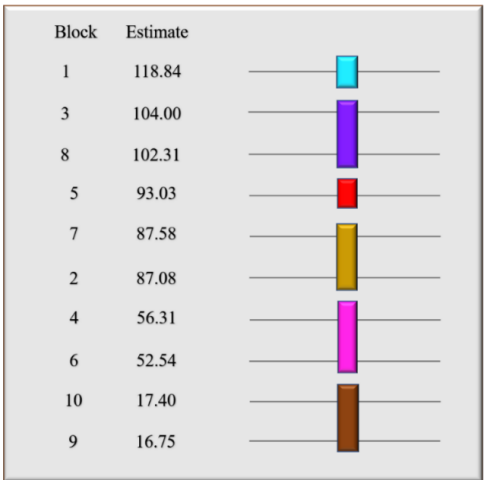

Figure 4: Statistical analysis data of TFC of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations)

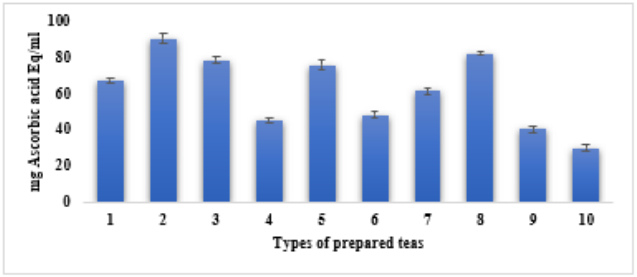

Figure 5: Comparison of total antioxidant capacity of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations)

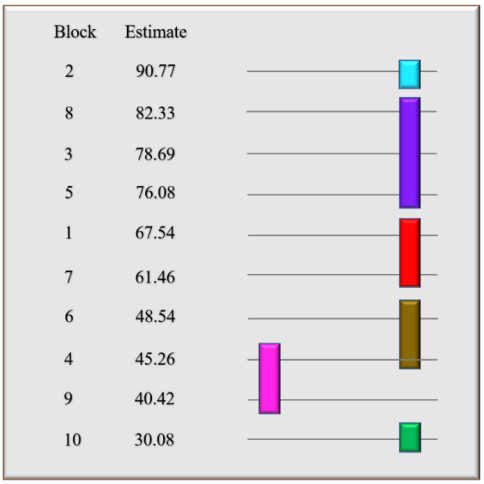

Figure 6: Statistical analysis data of total antioxidant capacity of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations)

1, while (Akila *et al*., 2018) reported that the TPC and TFC of guava leaf tea were 99.25 mg GAE/g and 13.29 mg QE/g, respectively. The statistical analysis (Figure 2) also revealed that those five prepared combinations of herbal teas have a higher TPC than the guava tea, and there are significant differences between each other at a 5% significance level. Figure 3 revealed that the guava tea has higher TFC than the prepared herbal tea, while the statistical analysis (Figure 4) also revealed the same. Although guava tea has a high TFC content, herbal teas also contain flavonoids, which can work well with polyphenols in herbal tea. As polyphenols and flavonoids are the key components of antioxidant properties, prepared herbal tea revealed high antioxidant properties. Particularly, the combination of guava leaf, cinnamon bark cinnamon leaf, avaram senna flower, gurmar leaf, holy basil leaf, and lemon leaf (35:20:1:1:1:1:1) exhibited the highest TPC and high antioxidant potential.

C. Antioxidant analysis

Antioxidant analysis of ten categories of herbal tea aqueous extracts using FRAP and DPPH assays revealed that the all-herbal teas exhibit antioxidant activity, which is shown in Figures 5 and 7. Despite the fact that all ten types of herbal tea extract exhibit antioxidant activity, the antioxidant capacity varies depending on the different combinations of medicinal plants in an herbal tea. In particular, total antioxidant capacity by FRAP assay confirmed that prepared tea 2 (herbal tea 1) has the highest reducing power (90.77 mg Ascorbic acid Eq/ml) of all the others, including guava tea (67.54 mg Ascorbic acid Eq/ml). Furthermore, statistical analysis also reveals the same, where the means covered by the same bar are not significantly different at the 5% significant level, which is shown in Figure 6. The DPPH radical scavenging effect investigation showed that prepared

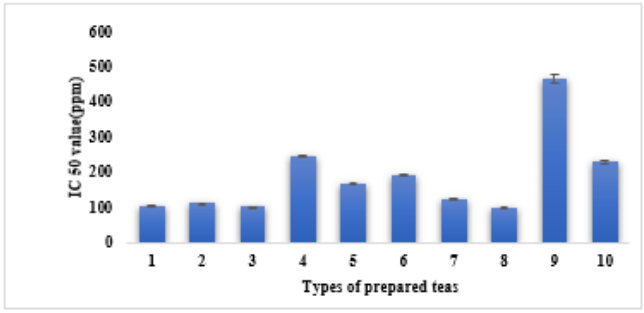

Figure 7: Effects of different organic additives on number of leaves per plantlet in orchid explant. Bars with similar letters are not significantly different

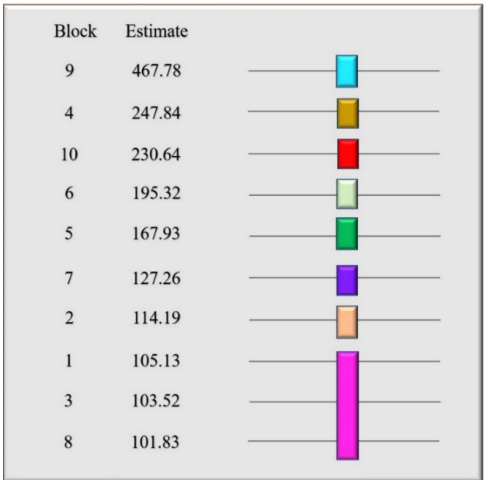

Figure 8: Statistical analysis of total radical scavenging activity of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations)

tea 8 (herbal tea 7) has a relatively high scavenging activity (IC_{50} value: 101.83 ppm) compared to the other prepared teas, including guava tea (prepared tea 1) (IC_{50} value: 105.13 ppm), while statistical analysis revealed that prepared teas 1, 3, and 8 had the same scavenging capacity at the 5% significant level as shown in Figure 8. From the results, prepared tea 8 (herbal tea 7) shows higher antioxidant activity due to its lower IC_{50} value.

D. Toxicity assay on brine shrimp

Herbal extract toxicity is frequently expressed as LC_{50} values and compared to Meyer's or Clarkson's toxicity indexes. Extracts with an LC_{50} of less than 1000 mg/L are toxic, while extracts with an LC_{50} of more than 1000 mg/L are non-toxic, according to Meyer's toxicity index. Thus, these results exhibit that guava and herbal teas are considered nontoxic due to their LC_{50} values higher than 1000 mg/L, which is shown in Figure 9.

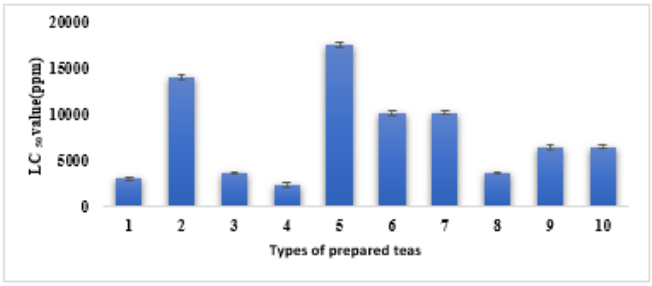

Figure 9: Comparison of toxicity of nine prepared herbal teas and guava tea (1: guava tea and 2-10: prepared herbal teas combinations)

Figure 10: Comparison of preference order of selected teas

Figure 10 displays that herbal tea 7 is most preferred (24 out of 30) by the semi-trained panel among the prepared teas due to their taste, color, smell, and texture, while guava tea is least preferred (27 out of 30) by them. Furthermore, herbal tea 1 is preferred second (16 out of 30). From the results, it reveals that herbal tea 7 is the most suitable and best tea to drink by people of all ages compared with guava tea.

The American Dental Association (ADA) reported that a pH between 2 and 4 is considered very dangerous to our teeth. Furthermore, black tea that the general public typically drinks has a pH range of 4.9 to 5.5, while Table II displays that guava tea and herbal tea 7 show the same pH value (5.06±0.01). From the results, herbal and guava teas are nowhere in that range. Therefore, the results revealed that guava tea and herbal teas 7 and 2 have a safe pH level to drink that will not cause tooth damage. Furthermore, herbal tea 7 (4.7±0.06 %) has the lowest moisture content compared to other teas, including guava tea (4.9±0.06 %). In comparison to guava tea, the results show that herbal tea 7 has a better pH level and moisture content.

Table II: Determination of physical properties

Type of selected teas	pH	Moisture content (%)
Guava	5.06±0.01	4.9±0.06
Tea 1	5.17±0.06	4.8±0.06
Tea 2	5.09±0.01	5.2±0.06
Tea 7	5.06±0.01	4.7±0.06

IV. Conclusions

This study revealed that the infusion of prepared tea bags was found to have phytochemical properties and antioxidant activity for the first time. We report the antioxidant and phytochemical properties of prepared herbal tea bags, which have been very popular for their medicinal properties, among

the rural community of Sri Lanka. The results of qualitative phytochemical analysis proved the presence of most of the phytochemicals in the prepared tea bags, except alkaloids. Thereby, phytochemicals may be responsible and further provide information regarding the antioxidant activity of these infusions. As polyphenolics and flavonoids are the key components of antioxidant properties, prepared herbal teas revealed antioxidant properties. In particular, the combination of guava leaf, cinnamon bark, cinnamon leaf, avaram senna flower, gurmar leaf, holy basil leaf, and lemon leaf (35:20:1:1:1:1:1) (Herbal tea 7) exhibited the highest TPC (2027.12 mg GAE/ml), total antioxidant capacity (82.33 mg Ascorbic acid Eq/ml) and free radical scavenging activity (101.83 ppm). Furthermore, toxicity assay and sensory evaluation preferred herbal tea 7, which has a better pH level and moisture content than guava tea. Antioxidants are responsible for many pharmacological properties. Hence, we can conclude that it would enhance the phytochemical properties of guava tea. Moreover, it would direct us to invent new and potent antidebate drugs of natural origin in the future.

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