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APPLIED BIO-SYSTEMS TECHNOLOGY

Research Article **Open Access**

Formulation and Characterization of Nutrient Bars using Underutilized Seeds: Semolina and Jackfruit Seeds

Janitha Sithari[1], Thilini Chandrasiri[1]* and Kolitha Wijesekara[1]

Abstract

Background: Fast foods are frequently used as an easy replacement of main meals by people, who lead busy lifestyles. Many nutrient bars are prepared with expensive and popular choices such as sunflower, flax, chia, sesame and hemp seeds. However, underutilized, cheaper alternatives such as semolina and jackfruit seeds could provide the same nutritional background required in a nutrient bar. This study was conducted to develop a nutrient bar from nutrient dense underutilized seeds as a main meal replacement.

Methods: Two distinct nutrient bars were formulated with watermelon seeds, winged bean, and pumpkin seeds as common underutilized seeds. Bar 1 (Treatment 1) was prepared by incorporating semolina seeds and Bar 2 (Treatment 2) was prepared incorporating flour of jackfruit seeds. Nutritional composition, physiochemical properties, microbial parameters and sensory profile of the formulated bars were determined using standard protocols suggested by the United State Department of Agriculture (USDA), Association of Official Agricultural Chemist (AOAC) standards, Sri Lanka Standards Institution (SLSI). The Friedman test was used for statistical analysis.

Results: Samples of Treatment 2 reported significantly highest moisture (14.4±0.66%) and fibre content (9.5±0.71%), while Treatment 1 had the highest fat level (10.1±0.01%). In terms of ash and protein content, there were no significant differences ($P>0.05$), between the developed samples and commercially available nutrient bars. Treatment 1 reported the highest calorific value (388.7 kcal/100g), while the highest phenolic content (8.3±0.30 mg GAE/g) and antioxidant activity were observed in Treatment 2. Based on Sensory evaluation, mean values of colour and mouth feel were highest in Treatment 2, while the mean values for aroma, texture, and overall acceptability were highest in Treatment 1.

Conclusions: Based on overall performance nutrient bar developed under Treatment 1 could be recommended as an excellent source of energy and nutritional component for a daily meal replacement.

Keywords: Daily Meal Replacement, Nutrient Bar, Natural Preservatives, Underutilized Seeds

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INTRODUCTION

Nutrients are compounds in foods, which are essential to maintain bodily functions and a healthy lifestyle. Food provides us with energy, acts as building blocks for repair and growth of cells and provides substances to regulate biochemical processes within the human body. There are six major nutrients: Carbohydrates (CHO), Lipids (Fats and Oils), Proteins, Vitamins, Minerals, and Water [1]. The amount of energy contained in food is indicated by the number of calories in that food. The recommended daily intake of calories depends on age, sex, and the level of physical activity. On average an individual needs to obtain around 2000 calories each day to maintain the basic bodily functions [2].

Many people who live a busy lifestyle tend to overlook the necessity of taking sufficient amounts of daily intake of nutrients coming from a balanced diet. Most people who engage in day jobs in public and private sector skip their main meals due to their busy work schedules [3]. Some find fast food as an alternative to replace the main meals, which could be consumed on the work. However, the majority of fast foods are incapable of providing the goodness coming from a balanced diet. On the other hand, most fast foods are high in calories and unhealthy saturated fatty acids. Regular consumers of fast food face the danger of contacting non-communicable diseases like diabetes and high blood pressure and often suffer from obesity due to high calorie uptake [4].

Nutrient bars could provide a solution for people, who often skip their main meal yet search for a suitable alternative to take daily recommended nutrients. A better formulated nutrient bar could provide a variety of essential macro and micro nutrients, as well as, sufficient amounts of protein and carbohydrates to keep the body running smoothly. Nutrient bars may concentrate on protein and reduced carbohydrates, or they may attempt to serve as a full meal with a higher caloric load (350 kcal), depending on the intent [5].

Most commercially available nutrient bars have been formulated by incorporating expensive ingredients such as sunflower, flax, chia, sesame and hemp seeds. Underutilized seeds, which are often discarded as agro-industrial waste by food and beverage processing companies could provide a cheaper alternative to replace expensive ingredients and at the same time provide the vital nutrients required from a nutrient bar. Production of well-balanced nutrient bar with a reasonable price tag could effectively cut down the consumption of unhealthy fast-foods [6].

This study attempts to develop a nutrient bar incorporating cheap and often wasted ingredients [7], such as Semolina which is a product of wheat milling. It is rich in dietary fibre, while seeds of jackfruit are rich in starch, calcium, vitamins, minerals and antioxidants [8]. Meanwhile, nutrient dense watermelon seeds are rich in protein and fibre [7]. Pumpkin seeds are a rich source of protein, fibre and minerals [8-9].

In previously reported studies; Nadeem *et al*. (2018) has combined dates with cereal and legumes to make a date bar [10], while Kumar *et al*. (2018) has created a protein bar with added spirulina for children suffering from malnutrition [11]. However, these products could not suffice the full nutritional requirements of a full meal. Use of often wasted seeds of agricultural produce to formulate a nutrient bar could bring in a commercial value to them and provide farmers the opportunity to increase their income.

METHODOLOGY

Study Setting

All the studies covered under this research were conducted at Uva Wellassa University, Badulla from 28th of September 2020 to 31st of May 2021.

Preparation of Dry Ingredients

Winged bean seeds, watermelon seeds, and pumpkin seeds were washed, dried and then roasted under low flame (45 – 82 °C) until they were light brown in colour. Then they were grounded to obtain a fine powder.

Preparation of Nutrient Bars

Precooked seeds, desiccated coconut, corn flour, semolina, jackfruit seeds flour and salt were combined in a stainless-steel mixing bowl and mixed well under low heat. Sugar caramel was prepared and vanilla flavour and citric acid were added to the mixture. The resulting mixture was molded out (2.5×2.5×4 cm) and packed in a heat sealable aluminium foil package. Treatment plan of nutrient bars is given in Table 1.

Table 1: Formulations of Nutrient Bars

Ingredients (in gram)	Treatment 1 (T 1)	Treatment 2 (T 2)
Semolina	160g	0g
Jackfruit seeds	0g	160g
Corn flour	40g	40g
Watermelon seeds	40g	40g
Pumpkin seeds	40g	40g
Winged bean seeds	80g	80g

Proximate Analysis

Moisture content was measured using a moisture analyzer (DW-110MW laboratory Halogen Moisture Analyzer, China). The ash Content (AOAC, 942), crude fibre (AOAC, 978.10), crude fat (AOAC, 2003.05), crude protein (AOAC, 2001.11) were measured using AOAC standard methods [12-13]. To measure the Carbohydrate content, the moisture, ash, fibre, fat and protein content were totalled and then reduced by 100. The formula that was used to calculate the carbohydrate content is as follows.

$$\text{Carbohydrate \%} = 100 - (\text{moisture \%} + \text{ash \%} + \text{crude fibre \%} + \text{crude fat \%} + \text{crude protein \%}) \quad (1)$$

Figure 1: Samples Obtained for Treatment 1

Figure 2: Samples Obtained for Treatment 2

Gross Energy Value

Gross energy values of nutrient bars were calculated using standard factors for energy in the form of kcal/g as 4, 9 and 4 kcal/g for protein, lipid and carbohydrate, respectively. The energy contents were summed up to result total or gross energy [14].

Physico-chemical Analysis

Water activity (a_w) was measured using AQUALAB 4TE Water activity meter, while pH value was measured with a portable pH meter after calibration. Brix determination was done using Mettler Toledo Refracto 30GS Portable Handheld Refractometer. Following calculations were used to calculate the Brix value (Equation 2 and 3).

$$\text{Degree of Factor} = \frac{1 + \text{ volume of water}}{\text{weight of sample}} \quad (2)$$

$$\text{Brix value} = \text{reading of refractometer} \times \text{ degree of factor} \quad (3)$$

Phyto-chemical Analysis

Antioxidant activity was measured using DPPH Radical Scavenging Assay [15]. One gram of each powdered nutrient bar sample was taken into a small beaker and 9 ml of distilled water was added into it to prepare the solution. From each prepared powder solutions, 1.2 g was taken and it was mixed with 20 ml of 80% methanol. The solutions were added into screw cap tubes and were centrifuged at 11,000 rpm for 10 minutes. Around 1 ml of 80% methanol was filled into a set of test tubes and 20 µl of the sample was transferred in to the first test tube, 40 µl sample was put into the second test tube and 60 µl sample was added into the third test tube. All the tubes were vortexed using a vertex mixer for 2 minutes. DPPH solution was prepared mixing 3.94 mg of DPPH powder with 100 ml of absolute methanol. From the sample, 0.5 ml was taken into another tube and 2.5 ml of DPPH solution was added into each tube. The test tubes were kept in a dark room for 20 minutes and absorbance was determined at 517 nm using a UV spectrophotometer (N-6000 Model, Yoke Instruments, China). The inhibition % was calculated using the following formula.

$$\text{Inhibition } \% = \frac{\text{control} - \text{ sample}}{\text{control}} \times 100 \quad (4)$$

Shelf-Life Evaluation

Total plate count of nutrient bars was determined according to the procedure given in SLS 516: part 1: 1991 [16]. Colony forming units were calculated using the following formula.

$$\text{Colony Forming Units/ ml} = \frac{\text{No. of colonies} \times \text{total dilution factor}}{\text{volume of culture plated in ml}} \quad (5)$$

Sensory Evaluation

Sensory characteristics of the nutrient bars such as colour, aroma, flavour, texture, taste and overall acceptability at room temperature were evaluated with a panel of 30 untrained panellists on a 9- point Hedonic Scale. The scale ranged from "Extremely Like" (1) to "Extremely Dislike" (9). During sensory evaluation a commercially available nutrient bar was used as the control.

Statistical Analysis

All the analysis were conducted in triplicate to verify accuracy of all results. The Mean ± Standard Deviation (SD) values were calculated for all the parameters, except for the sensory attributes. Data obtained from the proximate, physico-chemical, phytochemical analysis were subjected to the Analysis of Variance (One Way ANOVA), while the sensory attributes were subjected to Friedman analysis using Minitab 17. Significant differences of means ($P<0.05$) were further determined using the Tukey's pairwise comparison at a confidence level of 95%.

RESULTS AND DISCUSSION

Proximate Analysis

Results obtained for the proximate analysis of the nutrient bars are shown in the Table 2. The moisture content varied significantly amongst three types of nutrient bars ($P<0.05$ at 5% level of significance). The highest moisture content (14.4±0.66%) was observed in Treatment 2, while the lowest moisture content was reported from the commercially available nutrient bar (1.3±0.27%), as shown in Table 2. The chemical, physical, and microbial stability of foods are affected by the properties of water. Even a slight increment in moisture content of low and intermediate moisture containing foods can significantly reduce their shelf life. In addition, moisture content influences the textural properties of low moisture foods [17]. Therefore, the short shelf life of Treatment 2 could be related to its higher moisture content than the other samples.

There were no significant differences in the mean ash contents among the three types of nutrient bars (P>0.05 at 5% levels of significance). However, Treatment 2 had the high ash content (8.7±1.14%), which could be related to the presence of jackfruit seed flour that contain around 3087 mg/kg calcium, 130.74 mg/kg iron is, 1478 mg/kg potassium, 60.66 mg/kg sodium, 10.45 mg/kg copper, and 1.12 mg/kg manganese [18]. Generally, a high ash content means that the food product is a rich source of minerals [19].

The fibre content denoted significant differences among the three types of nutrient bars (P<0.05). Treatment 2 had the highest fibre content (9.5±0.71%), while the lowest fibre content was observed in the commercially available nutrient bar (0.5±0.71%), as shown in Table 2. Presence of high amount of dietary fibre makes it an excellent bulk laxative. The presence of high fibre content in jackfruit seeds flour prevents constipation and contributes towards smooth bowel movements [19].

The fat content denoted a significant difference among three types of Nutrient bar (P<0.05). The highest fat content was observed in the Treatment 1 (9.9±0.01%), while the lowest fat content was observed in the commercially available nutrient bar (1.7±0.01%). Treatment 2 contained 7.1±0.01% of fat. According to the United State Department of Agriculture (USDA), a nutrient bar should contain 10% (w/w) fat. The prepared nutrient bars in this study recorded the required amount of fat compared to the commercial nutrient bar. The Dietary Reference Intake (DRI) for fat in adults is 20% to 35% of total calories from fat. That accounts to about 44 g to 77 g of fat per day, if the total intake of calories per day is 2,000. It is recommended to eat more of monounsaturated fat (15% to 20%), polyunsaturated fat (5% to 10%) and less saturated fat (less than 10%), because they provide health benefits. Further, it is recommended to eat less of trans fat (0%) and cholesterol (less than 300 mg per day), due to the negative impacts on health [20].

The protein content did not change significantly in all three types of nutrient bars. The highest protein content was observed in Treatment 2 (29.5±3.26%), while Treatment 1 reported the lowest protein content (20.9±2.31%), as shown in Table 2. Main protein contributions are coming from the seeds used in the formulations. Reports indicated that Jackfruit seeds contain 13.50% protein [18], Pumpkin Seeds has 30.23% [21], Winged bean seeds contain 34.18-40.30% protein [8] and Watermelon seeds contain 16.33- 17.75% of protein [7]. Proteins are required for the growth and maintenance of tissues and could also serve as a valuable energy source, but only in situations of fasting, exhaustive exercise or inadequate calorie intake.

The carbohydrate content had significantly changed among three types of nutrient bars (P<0.05 at 5% levels of significance). A typical energy bar supplies 20-40% (w/w) of carbohydrate. The commercially available nutrient bar had 66.4±0.01% of carbohydrate. However, the prepared nutrient bars had the required quantity of carbohydrates. Generally, the nutrient bars that contain a concentrated source of carbohydrates for quick energy and a source of protein for muscle repair and growth are formulated to cater the needs of sports and fitness enthusiasts.

Gross Caloric Value

Caloric values of different nutrient bars were calculated as shown in the Table 3. According to the USDA [22], a nutrient bar provides 350 kcal/100 g. The Treatment 1 was reporting the highest amount of calories, indicating that it may be utilized as a meal replacement, because it provides sufficient energy for the human body, compared to Treatment 2.

Table 2: Proximate Analysis of the Nutrient Bars

Parameter	Treatment 1	Treatment 2	Commercially Available Nutrient Bar
Moisture	3.5±1.18^{b}	14.4±0.66^{a}	1.3±0.27^{c}
Ash	7.3±2.53^{a}	8.7±1.14^{a}	7.8±0.01^{a}
Crude fibre	4.5±0.71^{b}	9.5±0.71^{a}	0.5±0.71^{c}
Crude fat	9.9±0.01^{a}	7.1±0.01^{b}	1.7±0.01^{c}
Crude protein	20.9±2.31^{a}	29.5±3.26^{a}	22.5±0.71^{a}
Carbohydrate	53.9±0.00^{b}	30.9±0.01^{c}	66.4±0.01^{a}

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar. Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Table 3: Caloric Value of Different Nutrient Bars

Sample	Gross Energy Value (kcal/100g)
Treatment 1	388.6±0.00^{a}
Treatment 2	305.0±0.00^{c}
Commercially Available Product	370.1±0.00^{b}

Note: Treatment 1: Semolina incorporated Nutrient bar Treatment 2: Jackfruit seeds flour incorporated Nutrient bar. Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Physico-Chemical Analysis

Average values of physico-chemical characteristics of the three types of nutrient bars are shown in Table 4. For any sort of bacteria, the minimum a_w value required for growth is of 0.75, while osmophilic yeast and xerophillic fungi are capable to develop in a_w of 0.61 and 0.65, respectively. Therefore, commercially available nutrient bar presented a_w with values below 0.60, while Treatment 1 presented a_w value below 0.75. Treatment 2 reported a high a_w value than the Treatment 1. This could be the reason for the short shelf life obtained for Treatment 1 and 2 compared with the commercial product.

Foods without adequate acidity may allow the growth of microorganisms (bacteria, molds, parasites), which causes food spoilage and food-borne illnesses. Citric acid can be used to acidify the foods. Low acidic foods have the pH value greater than 4.5. For caramels it is in the 4.5 – 5.0 pH. Vegetables with a more neutral pH are in the 4.6 to 6.4 range [23]. Since the both prepared samples had been incorporated with the citric acids and caramels, Treatment 1 showed 5.74±0.09 as the mean pH value, while 5.83 ± 0.27 was reported as the mean pH of Treatment 2, which can be considered as low pH values, compared to the commercially available nutrient bar.

Sugar content is an important determinant of the nutritional value, since refined sugar acts as a quick and simple source of energy and provide taste characteristics of processed foods. The ability to rapidly measure sugar content during food production and processing is critical in ensuring consistent high product quality. Brix is a method that has been widely used to rapidly verify the sugar content [24]. The highest Brix value was shown in Treatment 1 (56.4±7.36%), while the lowest brix value was reported in Treatment 2 (51.9±7.73%). It did not denote significant difference among three types of nutrient bars (P>0.05 at 5% levels of significance).

Table 4: Physico-Chemical Characteristics of Nutrient Bars

Types of Nutrient Bars	Water Activity (a_w)	pH	Total Soluble Solids/ TSS (Brix) (%)
Treatment 1	0.70±0.06[a]	5.74±0.09[b]	56.4±7.36[a]
Treatment 2	0.76±0.03[a]	5.83±0.27[b]	51.9±7.73[a]
Commercially available nutrient bar	0.54±0.00[b]	6.89±0.02[a]	55.7±0.90[a]

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar. Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Phytochemical Analysis

In the present study, the total phenolic content of the Treatment 2 was reported the highest value compared with the other nutrient bars. Inclusion of jackfruit seeds could be the reason for this high phenolic content, since jackfruit seeds contain lignans, isoflavones, saponins, and many phytonutrients. The health benefits of these phytochemicals are wide-ranging from anti-cancer to anti-hypertensive. These antioxidants are also useful as anti-ulcer and anti-aging tonics [11].

The antioxidant activity denoted significant differences among three types of nutrient bars (P<0.05). The %DPPH inhibition measures the free radical scavenging property of a particular substance and is a measure of its antioxidant potential. The DPPH radical scavenging activity depends on the phenolic compounds present in the sample, and the samples that are rich in phenolics, exhibit high DPPH inhibition [18]. Treatment 2 showed a high antioxidant activity (low IC_{50} value).

Microbial Analysis

Microbial analysis was done in order to ensure the product is safe for human consumption throughout the storage period. Total plate count was detected at 7 day time intervals for 28 days of storage time, for nutrient bars along with the heat sealable aluminium foil package. The total Plate Count was lower than the standard limits given by SLSI (less than 1×10^4 CFU/g) for Treatment 1 for 21 days and for Treatment 2 for 14 days, without adding any artificial preservatives. The commercially available nutrient bar's shelf life was noted as one month in their label.

Sensory Evaluation

In terms of sensory attributes, the estimated median score for colour and mouth feel were highest in Treatment 2, but the estimated median score for aroma, texture, and overall acceptability were highest in Treatment 1.

Table 5: Phytochemical Analysis Results of Nutrient Bars

Types of Nutrient Bars	Total Phenolic Content (mg GAE/g)	Antioxidant Activity IC 50 (mg/ml)
Treatment 1	2.9±0.39[c]	350.7±5.49[a]
Treatment 2	8.3±0.30[a]	211.9±0.58[b]
Commercially Available Product	4.2±0.46[b]	219.6±1.33[b]

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar.
Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Table 6: Results of the Total Plate Count

Type	Just after Preparation (CFU/g)	After 3 Days (CFU/g)	After 7 Days (CFU/g)	After 14 Days (CFU/g)	After 21 Days (CFU/g)	After 28 Days (CFU/g)
Treatment 1	0	0	30	330	670	TMTC
Treatment 2	0	70	300	500	TMTC	TMTC

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar; TMTC: Too Much To Count.

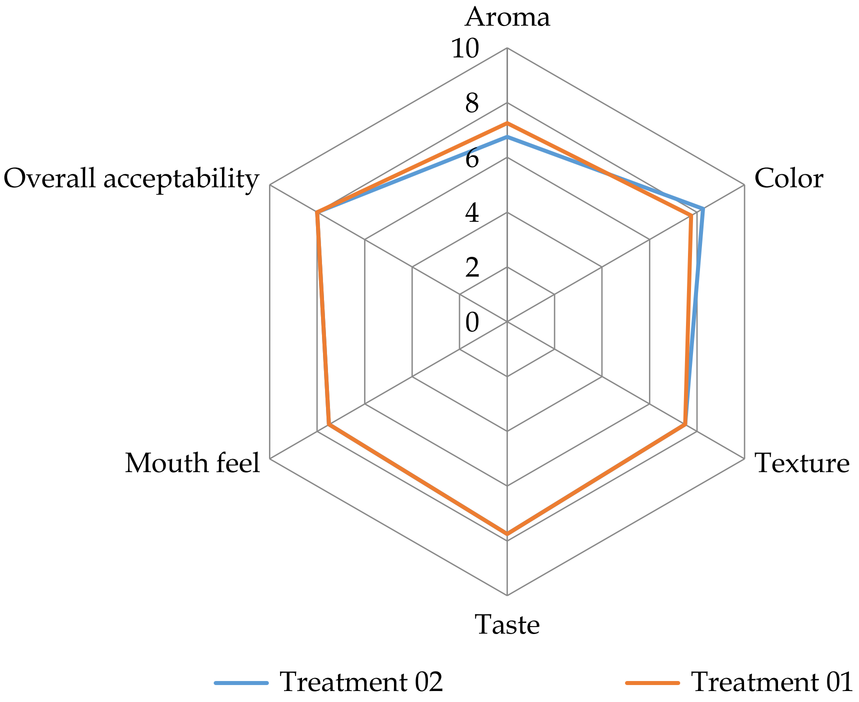

Figure 3: Spider-Web Diagram for Sensory Evaluation of Two Products

Table 7: Cost Analysis of the Developed Nutrient Bars

Ingredients	Price/ 100g of Mixture (T 1) / Rs:	Price/ 100 g of Mixture (T 2) / Rs:
Common raw seeds	50.00	50.00
Semolina	12.25	-
Jackfruit seeds	-	15.00
Sugar	2.00	2.00
Other ingredients (Salt, Glucose syrup, Citric acid Vanilla)	10.00	10.00
Others	20.00	20.00
Total	**94.25**	**97.00**

Note: T 1: Semolina incorporated Nutrient bar T 2: Jackfruit seeds flour incorporated Nutrient bar

According to the estimated median values, Treatment 2 had the highest value for colour while the Treatment 1 had the highest value for aroma. In case of the overall acceptability, Treatment 1 had the highest acceptance, compared with the Treatment 2.

Cost Analysis

Cost of production for a commercially available nutrient bar in Sri Lankan market is about Rs. 100.00 per 100 g. The processing cost of 100 g of nutrient bars developed from Treatment 1 and 2 were Rs. 94.25 and Rs. 97.00, respectively.

CONCLUSIONS

The developed nutrient bars meet the recommended dietary allowances, according to proximate analysis. Foods that fall within the meal replacement product compositional criteria should have energy contents between 200-250 kcal and 25.5% of that energy should come from protein, followed by 30–35% from fat. Therefore, the prepared nutrient bars met these requirements and ensure that both of these nutrient bars can be used as a meal replacement.

Product developed from Treatment 2 demonstrated a lower IC_{50} value (highest antioxidant activity), than Treatment 1 and the commercial nutrient bar. Meanwhile, the Treatment 1 had a longer shelf life (21 days) than Treatment 2 (14 days). Based on the sensory evaluation, Treatment 1 scored the highest mean values for overall acceptability. Moreover, nutrient bar developed using first treatment had the least cost of production based on the cost analysis. With the above scientific reasoning, nutrient bar developed using Treatment 1 could be commercialized as an effective, convenient, nutritional daily meal replacement substituting junk and fast foods.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

HJS: Carried out the investigations, data collection, statistical analysis, and prepared the first draft of manuscript; TC: supervised the study and revised the manuscript; KW: supervised the study. All authors read and approved the manuscript.

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APPLIED BIO-SYSTEMS TECHNOLOGY

Review Article Open Access

Recent Trends in Functional Foods and Nutraceuticals as Health-Promotive Measures: A Review

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Abstract

Non-Communicable Diseases (NCDs) have become a major health concern worldwide. The global death percentage caused by NCDs is reported to be 70% of the total deaths. Currently, there is a significant concern about herbal applications in improving people's lifestyles to mitigate the risk of NCDs, and food product development with an herbal context is considered more impactful. Plant/herbal materials have been used in traditional medicine since ancient times due to the nutraceutical properties of secondary plant metabolites. These are known to exert several health-promoting effects such as antioxidant, anti-cancer, anti-lipidemic, anti-hyperglycemic, etc. properties. Therefore, modern society is concerned more about adopting to pharmaceuticals and diet interventions of natural-origin to mitigate health conditions associated with NCDs. Those interventions are, in most cases, termed functional foods and/or nutraceuticals. Thus, a substantial global market opportunity has been relieved for herbal functional foods and nutraceuticals, recently. Therefore, this paper provides a narrative review on the global burden caused by the NCDs, and the deviation of consumer trends towards more natural and herbal oriented functional foods in overcoming those risks. Furthermore, such trends are predicted to rise drastically in upcoming years in the regions around the globe with significant generation of revenue. This review further elaborates on pharmacological and health benefits of herbal materials that could be used in developing functional foods and/or nutraceuticals. In addition, current and prospective functional foods and nutraceuticals that have been developed with herbal origins in recent research across the globe are presented here with their respective health-promoting effects. The food categories currently being developed into functional foods are mostly being, but not limited to, functional beverages, functional teas, functional snacks/starchy foods, and functional confectioneries. The physiological benefits expected by these functional foods and nutraceuticals include, prevention of hyperglycaemia, cardiovascular disease, hypertension, cancers, hypercholesterolemia, etc. This review would provide a brief but informative background for future researchers, who would carry out research on New Product Development (NPD) on functional foods and nutraceuticals of herbal origin.

Keywords: Functional Foods, Herbal, Non-Communicable Diseases, Nutraceuticals

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INTRODUCTION

There is a huge health concern all around the world despite the country's status on Non-Communicable Diseases (NCDs). With the advancement of science, much research has been conducted and several remedies were found to treat communicable or acute diseases such as infections to date. Pharmaceuticals play a vital role in this scenario. However, the global burden due to NCDs and chronic diseases captures the attention of many medical researchers as they are being responsible for more than 70% of the annual global deaths and due to lack of remedial measures. The fact that those diseases highly depend on food and lifestyle, and the uprising demand for foods and drugs of natural origin, current research trends are focused toward functional foods and nutraceuticals.

Global Burden of Non-Communicable Diseases

Globally, NCDs have been rising continuously in recent years, despite the countries or regions. As reported in 2010, in 2005, the NCDs caused about 35 million deaths around the world, which accounts for 60% of all deaths worldwide. It is estimated to grow by 17% within the next 10 years [1]. At present, the global death percentage is reported to be over 70% of all deaths and is around 41 million people per year, which even exceeds the expected rise of the death percentage according to the latest statistics published by the World Health Organization (WHO) in 2020 [2]. This regular increase in death rates caused by NCDs has influenced the United Nations to discuss “Non-Communicable Diseases” in three out of the very few high-level meetings on health-related issues on 19-20th September, 2011 [3], 10-11th July, 2014 [4] and 27th September 2018 [5].

NCDs are also known as chronic diseases and tend to be of long duration. These result from a combination of genetic, physiological, environmental, and behavioural factors [6]. Several health conditions are categorized as NCDs. According to previous researches, it has been determined that four major disease clusters, namely cardiovascular diseases, cancers, pulmonary diseases, and diabetes, are responsible for 80% of NCD-related deaths [3, 7]. Other than these, several other diseases that are not transmitted from person to person and do not cause acute infections that are classified as NCDs, such as asthma, digestive diseases, neurologic disorders, mental and behavioural disorders, kidney diseases, gynecologic disorders, hemoglobinopathies, sense-organ, and oral disorders, bear a significant burden [3]. The WHO has identified several risk factors for NCDs. Among them, depression, impaired glucose tolerance, high cholesterol, high blood pressure, obesity, unhealthy diet, smoking, physical inactivity, and excess alcohol consumption have been identified to be more critical [8].

Further, many systematic reviews have summarized the most influential risk factors of NCDs and, therefore, the strongest preventive factors cross-linked with the five major NCD categories (dementia, diabetes, stroke, coronary heart disease, and cancer) are unhealthy diet, physical inactivity, and smoking [3, 9]. As a healthier diet is a major preventive factor and/or risk factor for NCDs, a growing interest towards healthy food consumption has captured massive awareness throughout the globe. Plant-based products such as functional foods and/or nutraceuticals have emerged as a high-impact preventive measure for NCDs across the globe [7-9].

Functional Foods and Nutraceuticals

Functional foods and/or nutraceuticals are considered beneficial in preventing or treating diseases or improving health conditions, other than their usual nutritional value [10]. However, the terms “Functional foods” and “Nutraceuticals” have confusion in their definitions and are not defined well to separate between the two terms and also with terms such as “Conventional foods” and

"Pharmaceuticals". Thus, it has been given several similar, yet non-unified definitions in many research and review publications [11]. Generally, functional foods and nutraceuticals are considered to occupy a grey area between food and drugs [12]. The concept defining and initiation of these terms happened in the early 1980s by Japanese scientists, and the term "Nutraceuticals" was also formed in 1984 by Dr. De Felice by coining "Nutrition and Pharmaceuticals" [12-18].

Functional foods are defined by "Health Canada" as foods that resemble traditional foods, while demonstrating physiological properties. Meanwhile, nutraceuticals are derived from foods and presented in a non-food matrix but in medicinal forms such as capsules, tablets, and so on, and used to demonstrate physiological benefits in a concentrated form [10- 11, 19-22]. In many studies and writings, the definition of nutraceuticals is slightly varying. They are known as products with physiological benefits or those that provide protection against chronic diseases, derived from plant, animal, or marine sources or produced from processing plant material [18]. According to Pandey *et al.* [12], aside from the physiological benefits they provide, nutraceuticals are not traditionally recognized as nutrients, and Murthy [23] claims nutraceuticals are closely resembling drugs, except for their natural origin [12, 23]. However, there are still many definitions, which indicate nutraceuticals as foods or parts of foods [23, 24].

The European Union has suggested a working definition for functional foods in their Concerted Action on Functional Food Science in Europe (FuFoSE) as, when a food product has beneficial effects on one or more functions of the human organism; either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases along with its basic nutritional impact. However, this definition limits its consumption format and dosage to the conventional food types in usual dietetic proportions [13, 25, 26]. However, this is controversial with the Japanese definition derived for Food for Specified Health Use (FOSHU), including the forms of tablets and capsules too, where health claims or benefits remain a common requirement [13]. Japan was the first country to have functional foods and nutraceutical regulations, and Japanese food regulations for health claims have been greatly clarified. So far, over 200 products are in the market under this FOSHU category [10, 15, 27-29]. The USA does not have a well-defined and regulated framework for defining or claiming functional foods and nutraceuticals. They are either categorized as foods or drugs, and the products are usually regulated by the food laws of the United States [30].

Key Concepts of Functional Foods and Nutraceuticals

With this ambiguity of definitions, identifying the key properties of both functional foods and nutraceuticals is important in designing and developing novel functional foods/ nutraceuticals [31]. The major concepts covered by the definitions for both terms "Functional foods" and "Nutraceuticals" are summarized in a Venn diagram (Figure 1).

Apart from the above summarized key factors, Doyon & Labrecque [11] have identified four major concept areas covered by most of these definitions. Those are health benefits, nature of the food, level of function, and consumption pattern.

Health Benefits: Functional foods or nutraceuticals, have been identified and claimed for several health benefits in preventing, reducing the risk, or treating a disease and/or unfavourable health condition. Strong literature evidence was found on the ability of functional foods and nutraceuticals in preventing and treating several types of cancers in combination effects such as antioxidant activity, increased detoxifying enzyme activity, maintenance of DNA repair, and effects on cell differentiation

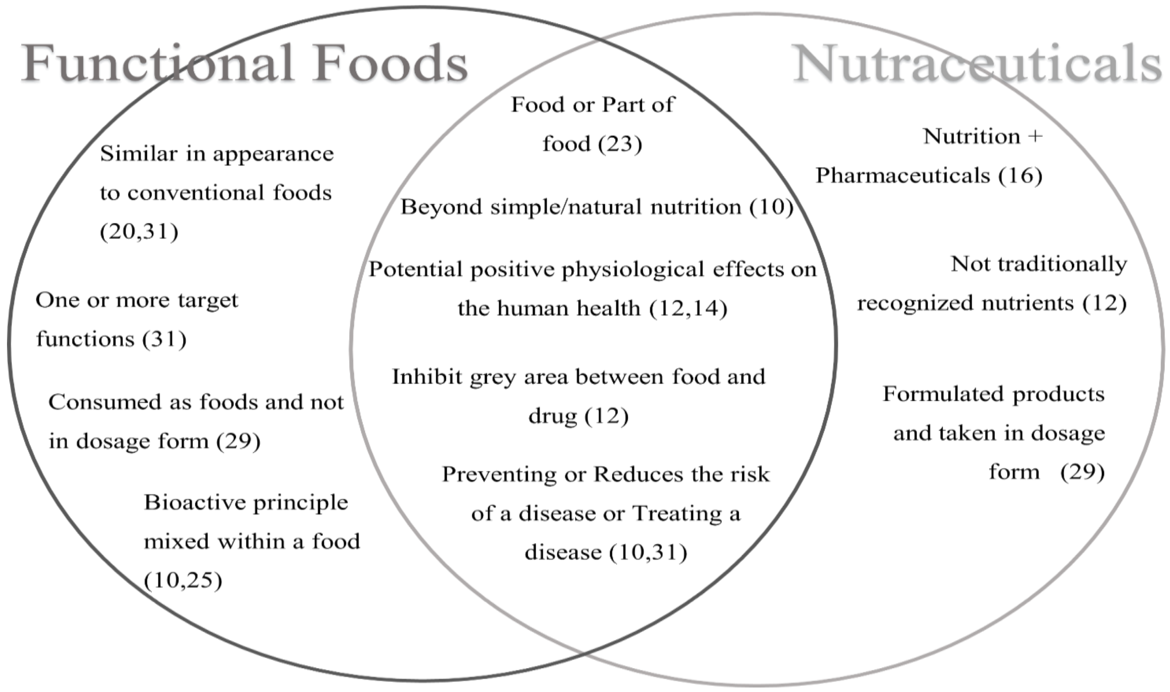


Figure 1: Key Concepts Covered by Many Definitions on the Terms, "Functional Foods" and "Nutraceuticals"

etc. [20, 32-34]. Cardiovascular diseases along with cross-linking health conditions; obesity, high blood cholesterol, high blood pressure, and type 2 diabetes, are supported by functional foods and nutraceuticals through many mechanisms including lowering blood lipid levels, decreasing plaque formation, reducing lipoprotein oxidation, improving arterial compliance, scavenging free radicals, and inhibiting platelet aggregation [20, 35-37]. Continuous consumption of plant-derived functional foods is supportive in preventing and managing type 2 diabetes with their antioxidant, anti-inflammatory, insulin sensitivity, and anti-cholesterol functions due to the content of nutraceuticals including; polyphenols, terpenoids, flavonoids, alkaloids, sterols, pigments, and unsaturated fatty acids [38-40].

Nature of Food: There is no box-framed definition for functional foods and nutraceuticals and the confusion is caused by aligning terms such as designer foods, dietary supplements, enhanced foods, etc. with functional foods. The nature of the food was also not defined without conflicts [41, 42]. Shinde *et al.* [43] categorized nutraceuticals in three major arenas regarding their nature, where dietary supplements are vitamins and minerals or botanicals like Ginseng, Gingko Biloba, Saint John's Wort, etc., functional foods to be a part of the usual diet with effects beyond traditional nutrition; and medicinal foods to be used in preventing or treating disease [43]. Although in general, functional foods are considered to look like conventional food and nutraceuticals to be in drug-like form, this might change from one product to another [29].

Level of Function: Functional foods or nutraceuticals are being used in several stages of functionality, where they could either be enhancing healthiness, preventing the occurrence of a disease or illness, reducing the risk of such illness, or even treating the diseases or illnesses. The benefits of functional foods and nutraceuticals may therefore include increasing the health value of the diet, increasing life expectancy; avoiding unfavourable medical conditions, imparting physiological benefits, being more natural with fewer side effects; and catering to special needs for dietary complexities such as celiac, lactose intolerant, or elderly patients [42].

Consumption Pattern: Functional foods and nutraceuticals are consumed similarly according to some limitations as they are food and/or part of food [23]. Furthermore, some consider a functional food to be one developed with scientific intelligence; when the functional food aids in the prevention or treatment of a disease other than anaemia, it is referred to as a nutraceutical [12, 16]. Also, the dosage or the consumption portions are different from each other as some definitions find nutraceuticals to be in dosage specified form while functional foods are to be taken in usual dietetic portions [29].

Nutraceutical Properties of Herbal Material

Traditional medicine, or folk medicine, has been used for supporting and curing NCDs since ancient times. The major active ingredients used in such medicinal approaches are herbal materials [44]. Although the available and recognized herbs vary region-wise, the utilization of herbals in NCDs has become prominent and captured global attention [45].

Numerous research and reviews have been carried out to identify the herbs and active compounds of herbs that are helpful in supporting many health conditions, which could ultimately be used in developing functional foods and/or nutraceuticals.

Moreover, it is evident that some herbal components are acting as functional foods even in their pure form and could be consumed as it is or with minimal processing and with an evident beneficial physiological impact. Examples: Berries, Cinnamon, Turmeric, Ginseng, Tomatoes, Soybeans, Oats, Psyllium, Flaxseed, Garlic, Grapes, and some nuts etc. [35, 39, 46]. Apart from these, there are many examples of products which incorporate refined functional compounds from plant derivatives [47, 48] and combined functional foods that show enhanced functionality or synergic effects [49, 50].

Types of Herbal Functional Compounds Present in Functional Foods /Nutraceuticals

A vast array of biologically active secondary metabolites take part in plant-derived functional foods and nutraceuticals.

- *Polyphenols:* These are a class of plant secondary metabolites that contain over 8000 compounds and protect plants from UV radiation, oxidants, and infections. They are found in whole plant foods such as fruits, vegetables, whole grains, cereals, legumes, tea, coffee, wine and cocoa [51, 52]. The main classes of polyphenols include phenolic acids, flavonoids, stilbenes, and lignins [51]. Epidemiological studies and associated meta-analyses in recent history strongly suggest that long-term consumption of a polyphenol-rich diet supports in management of many NCDs including cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [51, 53, 54]. Cardioprotective effect [55-57], anti-cancer effect [58], anti-diabetic effect [59], and neuroprotective effects [60-63] are some major beneficial effects of these polyphenols in human health.

- *Isoflavonoids:* These are also a class of phenolic phytochemicals that includes the major compounds genistein and daidzein. This class is recognized for preventing and treating cancer and osteoporosis. In particular, Genistein imparts cancer-preventive activity against colon cancer in vitro. The most predominant dietary source of isoflavones is Soybeans [64, 65].

- *Phytoestrogens:* This is a nonsteroidal phytochemical group that has a quite similar structure and function to the oestrogen hormone. And phytoestrogens show antioxidant properties due to their polyphenolic structure and anti-carcinogenic properties due to steroid metabolism or enzyme detoxification. Furthermore, they have a favourable effect on calcium transportation and lipid profiles [64, 66, 67].

- *Terpenoids:* These compounds also show cancer-preventive properties and are the largest class of phytonutrients found in green foods and grains. The most studied terpenoids are tocotrienols and tocopherols, commonly found in whole grains [65].

- *Carotenoids:* The Carotenoids are pigmented phytonutrients that can be divided into two major groups: carotenes and xanthophylls. Carotenes provide protection against uterine, prostate, breast, colorectal, lung, and digestive tract cancers, while xanthophylls provide protection for other antioxidants and offer tissue-specific protection. This subclass of phytochemicals is common in yellow-red fruits and vegetables [64, 68, 69].

- *Phytosterols:* An important subclass of phytonutrients. They are beneficial mostly in managing cholesterol levels in humans, as these compounds compete with cholesterol in the gut to eliminate cholesterol from the body without absorption [64, 70].

- *Glucosinolates:* This is another cancer-preventive phytochemical class and are present in cruciferous vegetables such as broccoli and cauliflower sprouts [64].

- *Polysaccharides:* These comprise of a large group of phytochemicals that provides many characteristics in food production like thickening, binding, bulking, etc. and are present in mushrooms, fruits and vegetables, legumes, cereals, and grains like oats and barley. These are responsible for many physiological functions, such as preventing cancer, obesity, and cardiovascular disease, normalizing blood pressure, balancing blood sugar levels, etc. [64, 71].

Herbs with Nutraceutical Potential

Listed below (see Table 1) are some herbs with potential nutraceutical properties due to their bioactive secondary plant metabolites and their respective health benefits. Other than the few summarized in Table 1, a strong herbal profile is important in supporting NCDs or chronic diseases, where some are still underutilized and under-recognized.

Biologically relevant compounds in action for reducing the risk of NCDs and treating NCD conditions are phytochemicals present in herbs, where the antioxidants capture the highest capacity and importance, as most chronic diseases, such as cardiovascular diseases, are associated with a high level of oxidative stress and could be managed through the antioxidant activity of plant compounds [42, 124]. Table 2 shows some important and recognized functional (bioactive) compounds derived from herbal materials [47, 99, 125–129].

Product Development of Functional Foods and Nutraceuticals

Global Market and Demand

The development of functional foods or nutraceuticals is considered to attract a long-term market value as their demand rises by 6-10% every year [11, 43]. Additionally, functional foods are typically priced 30-500% above comparable conventional foods [130]. According to the data taken in 2008, the global functional food market falls into the range of EUR 30-60 billion. It is reported that the functional foods and nutraceutical market in the United States were 250 billion US dollars in 2014 [43]. Although the European market is less developed compared to the USA and Japan, it still has a functional food market that

Table 1: Herbal Materials and their Secondary Metabolites that Impart Nutraceutical Properties

No	Potential Herb	Active Compounds	Supporting NCDs/ Beneficial Action
1	Aloe vera *Aloe barbadensis*	Aloins Aloesin	Dilates capillaries [72], Anti-inflammatory effect [72-77], Anti-diabetic effect [72-77]
2	St John's-wort *Hypericum perforatum*	Hypericin Hyperforin	Antidepressant effect [78, 79]
3	Turmeric *Curcuma longa*	Curcumin	Anticancer effect [24], Anti-inflammatory effect, Antiarthritic effect [80, 81]
4	Ginger *Zingiber officinale*	Zingiberene Gingerols Paradols Shogaols Zingerone	Anti-hyperglycemic effect, Antioxidant activity, Anti-inflammatory effect, Analgesic activity, Cancer preventive ability [82-86]
5	Ginseng *Panax ginseng*	Ginsenosides Panaxosides	Anti-tumour activity [87-89], Anti-diabetic effect [24], Enhanced liver function, Stimulating immune and nervous system [87-89]
6	Garlic *Allium sativum*	Alliin Allicin	Anti-inflammatory effect, Antigout effect, Antithrombotic effect, Hypotensive activity, Antihyperlipidemic effect [90-92]
7	Onion *Allium cepa*	Allicin Alliin	Hypoglycemic activity [24, 93], Antiatherosclerosis effect [75]
8	Gingko/ maidenhair *Ginkgo biloba*	Ginkgolide Bilobalide	Antioxidant activity, Increasing peripheral blood flow [94- 95], Treatment of post-thrombotic syndrome [89]
9	Valerian *Valeriana officinalis*	Valerenic acid Valerate	Tranquillizer effect [24, 96]
10	Liquorice *Glycyrrhiza glabra*	Glabridin Glycyrrhizin Liquirtin	Anti-inflammatory effect, Expectorant, Antioxidant activity, Hepatoprotective effect, Anti-carcinogenic activity [97- 99]
11	Gotu kola *Centella asiatica*	Asiaticoside Madecassoside	Nervine tonic, Anti-anxiety effect, Anticancer activity [100]

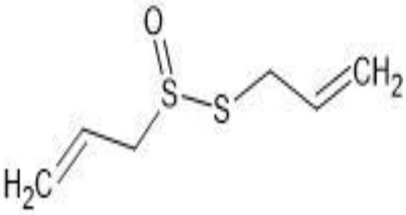
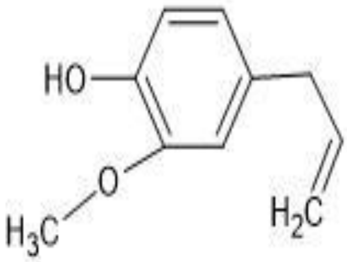
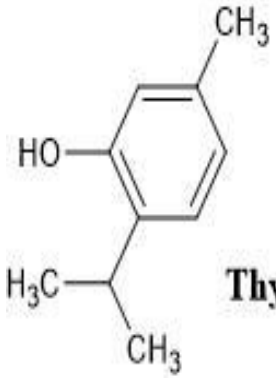
Table 1: Herbal Materials and their Secondary Metabolites that Impart Nutraceutical Properties
(Table 1 Continued)

No	Potential Herb	Active Compounds	Supporting NCDs/ Beneficial Action
12	Purple coneflower *Echinacea purpurea*	Alkylamide Echinacoside	Anti-inflammatory effect [101], Immunomodulator [102]
13	White willow *Salix alba*	Salicin	Anti-inflammatory effect, Analgesic, Treatment of rheumatic and arthritis [103-105]
14	Goldenseal *Hydrastis canadensis*	Hydrastine Berberine and Canadine	Antihemorrhagic [104, 106]
15	Quinoa *Chenopodium quinoa*	Gallic acid and other polyphenols Saponinshas Flavonoids Lysine Amino acid Phytosterols Phytoecdysteroids	Antioxidant activity, Anti-obesity, Hypocholesterolemic, Managing malnutrition, Anti-hyperglycemic [107-109]
16	Lavender *Lavandula spica L.*	Tannins	Cure depression and hypertension [76] Support Asthma [42]
17	Parsley *Petroselinum cripsum*	Apiol	Diuretic activity [76, 110]
18	Olive *Olea europaea*	Hydroxytyrosol, Oleuropein Tyrosol Oleuropein Aglycone	Antioxidant activity [111]
19	Rooibos *Aspalathus linearis*	Aspalathin and other polyphenols	Antioxidant activity, Hepatoprotective activity, Lipid-lowering effect Antidiabetic effect [112, 114]
20	Pumpkin *Cucurbita maxima*	Beta carotene	Antioxidant activity [115]
21	Guava *Psidium guajava*	Lycopene	Antioxidant activity [48, 116-118], Anticancer effect, Antihyperglycemic effect, Antihyperlipidemic effect [116-118]

Table 1: Herbal Materials and their Secondary Metabolites that Impart Nutraceutical Properties *(Table 1 Continued)*

No	Potential Herb	Active Compounds	Supporting NCDs/ Beneficial Action
22	Grapes *Vitis vinifera L.*	Resveratrol	Antioxidant activity, Antihypertensive effect [119]
23	Cinnamon *Cinnamomum verum*	Barely reported	Anti-diabetic effect [120]
24	Long Coriander *Eryngium foetidum*	Phenols, Flavonoids, Tannins, Polyphenols	Analgesic effect, Hypotensive effect, Anti-convulsant effect, Hepato-protective effect, Antioxidant activity [122, 123]

Table 2: Few Recognized Plant-Derived Active Compounds

 **Allicin**	 **Eugenol**	 **Thymol**
Source: *Garlic* ***Uses:*** *Protection against atherosclerosis, diabetes, high blood pressure, cholesterol*	***Source:*** *Cinnamon, Clove* ***Uses:*** *Antioxidant, gentle anaesthetic, and antiseptic activities*	***Source:*** *Thyme* ***Uses:*** *Helps in oral health, cancer-preventive, supports cardiovascular disease*

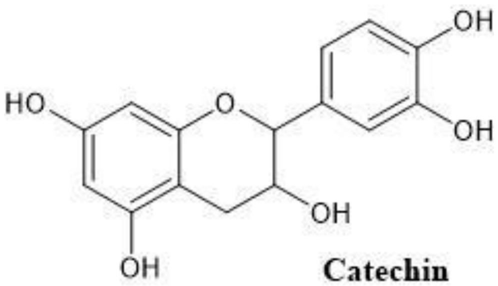
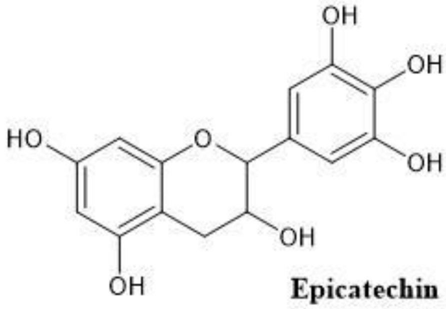
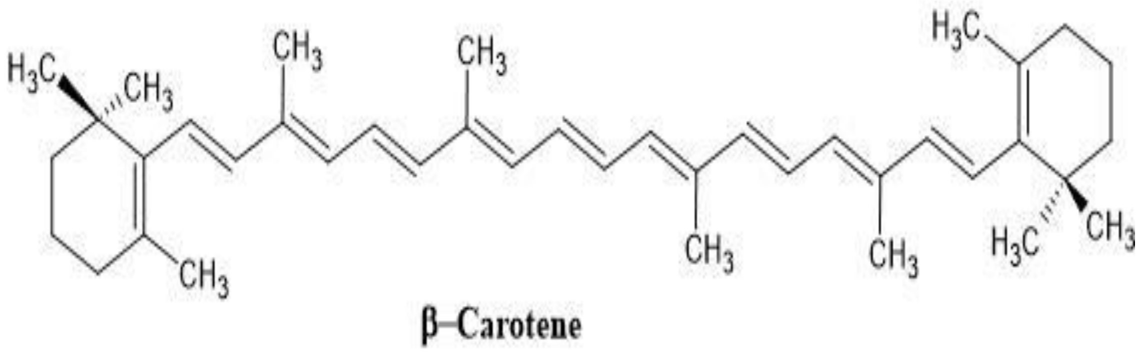
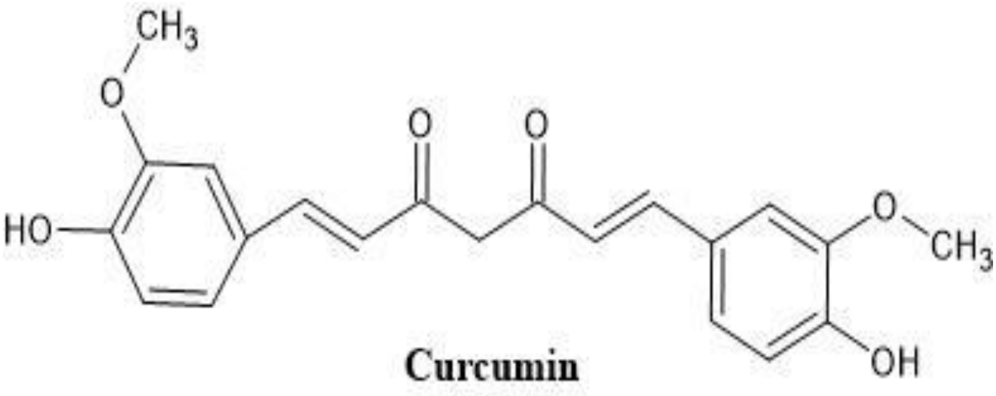
 **Catechin**	 **Epicatechin**
Source: *Tea and Pome Fruits* ***Uses:*** *Antioxidant, Anticarcinogenic, Antidiabetic, Antiatherogenic*	***Source:*** *Green tea, Dark Chocolate* ***Uses:*** *Antioxidant*

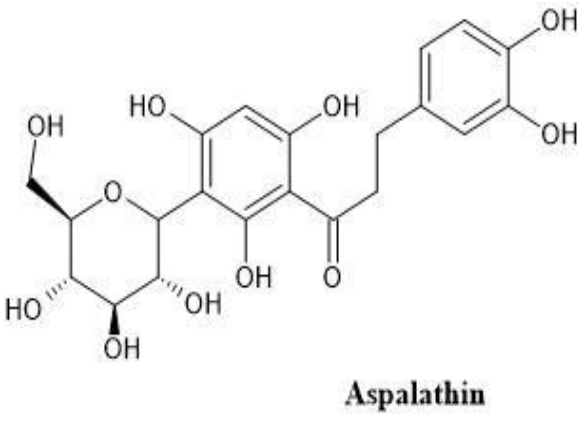
Table 2: Few Recognized Plant-Derived Active Compounds
(Table 2 Continued)

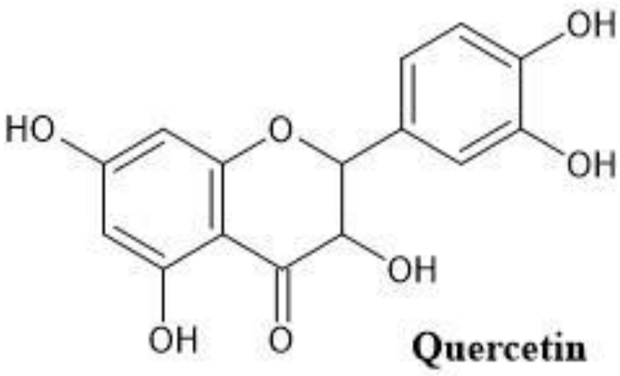


Source: *Yellow – Red Fruits and Vegetables*
Uses: *Antioxidants, Protection against Cancer, Heart disease, Alzheimer's disease*



Source: *Turmeric*
Uses: *Antioxidant, Protection against heart disease, brain disease, cancer, arthritis, and depression*

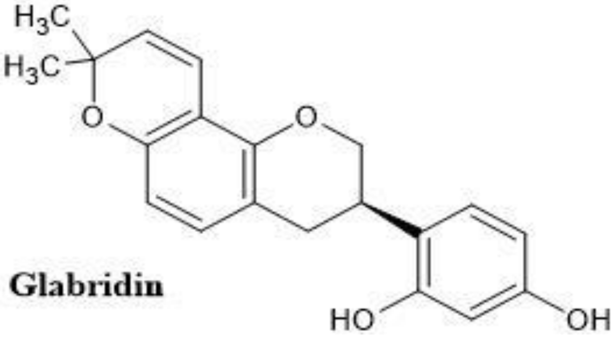




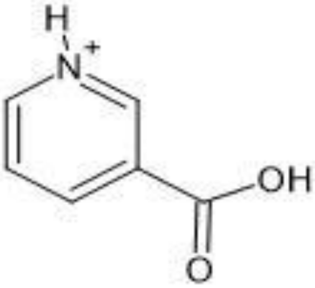
Source: *Rooibos*
Uses: *Antioxidant, Antidiabetic*

Source: *Apples, Berries, Grapes, Onion*
Uses: *Antioxidant, reducing cancer risk, neuroprotective, preventing cardiovascular disease*

Table 2: Few Recognized Plant-Derived Active Compounds
(Table 2 Continued)



Glabridin



Trigonellin

Source: *Liquorice* ***Uses:*** *Antioxidant, Hepatoprotective, Anticarcinogenic, Anti-inflammatory*	***Source:*** *Fenugreek, Japanese Radish, Oats, Potatoes* ***Uses:*** *Hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumour*

countries show tidal growth in the nutraceutical market [41, 43, 130]. Although the existing market is smaller, China (6 billion US dollars per year), Brazil (1.9 billion US dollars in 2009), and Russia (75 million US dollars in 2004) also showcase a growing market for functional foods [130]. Most recent reports states that, at a compound annual growth rate (CAGR) of 5.7%, the worldwide functional food market is anticipated to increase from $161.99 billion in 2020 to $171.25 billion in 2021 [131]. Companies adopting the new normal condition after the covid – 19 impact has been influenced in this growth while it is expected to rise at 8% CAGR to reach $228.79 billion in 2025 [131, 132].

Product Development

With the rising demand for functional foods and nutraceuticals, product developments with commercial feasibility play a useful role, facilitating both medicinal and economic aspects. However, the production of functional foods or nutraceuticals has penetrated only a very small area of its potential capacity.

According to several definitions and categorizations, functional foods and nutraceuticals may also include prebiotics and probiotics, enhanced products with omega fatty acids, functional meat products, algae or seaweed-based products, etc. [10, 13].

In this review article, the focus was given only to the plant/herbal-based product development of functional foods and nutraceuticals. However, even with the ample worldwide herbal profiles, there are only a few types of functional foods and/or nutraceuticals that have been reported in previous studies. Some examples are summarized in the below table (see Table 3) with their respective health-promoting activities.

As per the referenced products, the beverage production was the most outstanding. Moreover, bakery products, dairy products, confectionaries, tablets, capsules, and supplements with herbal incorporation were also considered in developing functional foods and nutraceuticals.

In addition to single herbal products, some researchers have focused on developing significantly impactful functional foods and nutraceuticals for specific chronic diseases with combinations of several plants and herbal materials. For example, Wijaya *et al.* [156] have developed a functional drink with

Table 3: Some Product Developments with Functional Food and/or Nutraceutical Properties from Previous Literature

No	Herbal Material	Plant Parts in Use	Food Type	Functional Interest	Reference
1	Rooibos *Aspalathus linearis*	Leaves	Tea Beverage	-	[133]
			Rooibos Tea	Antioxidant activity	[134]
				Antidiabetic activity	[127]
2	Quinoa *Chenopodium quinoa*	Grains	Flour, Biscuits, Pasta	Anti-diabetic properties	[135] [107]
3	Tea *Camelia sinensis*	Leaves	Green Tea	Reduce cholesterol, Raise energy, Stimulate mental power, Reducing the risk of coronary artery disease	[136] [137]
4	Java Tea (Cat whiskers) *Orthosiphon Stamineus*	Leaves	Java Tea	Antiallergenic effect, Antihypertensive activity, Anti-inflammatory effect, Diuretic properties	[136]
5	Roselle (Hibiscus) *Hibiscus sabdariffa*	Flowers	Functional Beverage		[138]
6	Lemon-Lime *Citrus limon, Citrus aurantiifolia*	Fruit	Frozen dessert	Nutritious supplement	[139]
7	Star fruit *Averrhoa carambola*	Fruit Juice	Functional beverage		[140]
8	Mushrooms *L. edodes*	Paste	Functional noodles	Antihypercholesterelemic activity	[141]
9	Pumpkin *Curcurbita maxima*		Fermented functional beverage	Supports Diabetes	[142]
10	Licorice *Glycyrrhiza uralensis*	Extract	Herbal Lollipop	Reduce dental cavities	[143]
11	Ginger *Zingiber officinale*		Ginger honey candy		[144]

Table 3: Some Product Developments with Functional Food and/or Nutraceutical Properties from Previous Literature *(Table 3 Continued)*

No	Herbal Material	Plant Parts in Use	Food Type	Functional Interest	Reference
12	Mate *Ilex paraguariensis* A.St.-Hil	Leaf Extract	Fermented functional beverage	Antioxidant activity, hypocholesterolemic and hepatoprotective effect	[145]
13	Tulshi *Ocimum sanctum* Moringa *Moringa oleifera*	Leaves	Herbal Biscuits		[146]
14	Pomegranate *Punica granatum*	Encapsulated peel phenolic	Ice cream	Antioxidant activity and a- glucosidase inhibitory activities	[147]
15	Guava *Psidium guajava*		Guava Cheese (fruit snack)	Antioxidant activity	[148]
16	Moringa *Moringa oleifera*	Leaves	Herbal tea		[149]
17	Kachai Lemon *Citrus jambhiri*	fruits	Squash, salt pickle, sweet pickle, candied peel, candied fruit slices, jelly	Nutritious supplement	[150]
18	Guava *Psidium guajava*	Leaf	Noodle or bread	hyperlipidaemic measures and supports high blood sugar level syndrome	[151]
19	Coffee *Coffea arabica L.* Siberian ginseng *Eleutherococcus senticosus* organic oat *Avena sativa* organic Gotu kola *Centella asiatica* Sassafrass *Sassafras albidum*	Beans Flower pollen Straw Leaves Leaves	Energy drink	Boosting energy	[152]

Table 3: Some Product Developments with Functional Food and/or Nutraceutical Properties from Previous Literature *(Table 3 Continued)*

No	Herbal Material	Plant Parts in Use	Food Type	Functional Interest	Reference
20	Rtanique fruit (a hybrid of tangerine and sweet orange) (*Citrus sinensis*×*Citrus reticulata*)		Apple snack	Antiradical capacity	[153]
21	Kyoho *Vitis labruscana*	Skins	Herbal tea	Antioxidant activity	[154]
22	Parsley *Petroselinum cripsum*		Pasta		[110]
23	Heartwood *Caesalpinia sappan*	Wood	BRE* Nutraceutical	Antioxidant activity, Antibacterial effect, Anti-inflammatory effect	[155]
24	Cocoa *Theobroma cacao*	Beans	Cocoa and Chocolate	Preventing Cardiovascular disease	[37]
25	Grapes *Vitis vinifera*	Skins	Wine	Antioxidant activity, Antihypertensive effect	[119]

a combination of Cat's whiskers (*Orthosiphon aristatus*), ginger (*Zingiber officinale*), Roscoe Lily (*Roscoea purpurea*), lime (*Citrus aurantiifolia*), lemon (*Citrus limon*), kaffir lime (*Citrus histrix*), and Curcuma (*Curcuma xanthorrhiza*) with anti-hyperglycemic effects [156, 157]. Another functional beverage with cistus (*Cistus incanus*), green tea (*Camellia sinensis*), nettle leaves (*Urtica dioica*), artichoke (*Cynara scolymus*), Siberian ginseng (*Eleutherococcus senticosus*), ginger (*Zingiber officinale*), purple coneflower (*Echinacea purpurea*), Aloe (*Aloe vera L.)* , horsetail (*Equisetum arvense*), lingonberry (*Vaccinium vitisidaea*), silver birch (*Betula pendula*), and chamomile (*Matricaria chamomilla*) was developed by Skąpska *et al.* [158] to yield higher antioxidant capacity [158].

Most of these studies are limited to the laboratory-level, and therefore, it is required to expand them to the commercial level. Further, there are many novel product developments at the research/experimental level, and even in the established market that is not published on scientific platforms due to trade secrets, pending patents, unproven health claims, and many other reasons.

Given the assessed and identified increasing global demand for functional foods and nutraceuticals, it is clear that commercial production of the aforementioned products, as well as novel research on the same, would benefit the community by lowering the medical costs while earning more economic benefits.

Physiological Benefits of Functional Foods/Nutraceuticals

Among the physiological benefits to human health imparted by functional foods and nutraceuticals, a wealth of literature provides evidence on preventing or treating non-communicable diseases with functional food or nutraceutical applications [115, 126].

Type 2 diabetes, or hyperglycaemia, is a predominant health concern that has a potential for prevention and management with the use of functional foods and/or nutraceuticals and their bioactive compounds [59]. Diets with high phytochemical content, high antioxidant capacity, and polyphenolic content act on lowering the risk of diabetes and predisposing factors [59, 159]. Another finding, which defines functional foods or nutraceuticals as individual bioactive chemicals or foods claimed to have health-promoting properties, provides several such applications in managing and preventing type 2 diabetes [160]. Predominant herbal applications, namely, American ginseng, Chinese herbs, Fenugreek, and Nopal, are some of the given examples in this context.

Nutraceutical compounds such as resveratrol from grape wine, curcumin from turmeric, cocoa, quercetin, brassica, and berberine were found to be beneficial in the prevention of cardiovascular diseases and their associated health conditions, hypertension, atherosclerosis, heart failure, and diabetes [119].

Many nutraceuticals are in action towards the treatment of cancers [64, 126]. Among these dietary supplements (Eg- Punicagrantum, Triticumaestivum, Beta vulgaris), plant secondary metabolites (Eg- flavonoids, phenols, carotenoids, alkaloids, saponins, tannins, steroids) and medicinal herbs (Eg- *Cynara cardunculus, Azadirachta indica, Santalum album*) play a significant role in colon cancers [126]. Ginger is one of the major potential ingredients in the form of water extract, paste, or dried powder in many functional food products proven to inhibit the progression of prostate tumors [161].

CONCLUSIONS

This review summarizes the recognition of functional foods and nutraceuticals as potential natural remedies for mitigating the global health risk of NCDs. According to recent research findings, the important pharmacological properties such as antioxidant, anti-inflammatory, anticancer, anti-hyperglycaemic, anti-lipidemic, and so on exerted by plant secondary metabolites, prospective and developed functional foods and nutraceuticals of herbal origin are narrated. The review concludes that developing easily adoptable technologies for producing functional foods and nutraceuticals would support the community in adopting healthier dietary patterns and promoting such plants as economical crops would thereby obtain both medicinal and economic gains for modern society

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

AUTHORS' CONTRIBUTIONS

UB: Conceptualized the study and wrote the manuscript. NL and VPB: Reviewed the manuscript. All authors read and approved the manuscript.

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APPLIED BIO-SYSTEMS TECHNOLOGY

Research Article **Open Access**

Evaluation of the Effect of Morphological Traits on Blister Blight Resistance in Tea Plant (*Camellia sinensis* L.)

Thissa Karunarathna[1], Mewan Kooragoda[2], Lahiru Udayanga[2], Jagathpriya Weerasena[3], Chandrika N. Perera[4] and Nishantha Edirisinghe[5]

Abstract

Background: Blister Blight (BB) is a serious leaf disease caused by the fungus *Exobasidium vexans* Masse, damaging Sri Lankan tea plantations.

Methods: A morphological trait-based analysis was preformed based on 14 descriptors for Camellia sinensis to differentiate BB resistant and BB susceptible individuals in anF1 population generated by a cross between BB resistant and BB susceptible cultivars (TRI 2043xTRI 2023). The Spearman's correlation analysis, regression modelling, Receiver Operating Characteristic (ROC) and t-test were applied in the analysis of morphological characteristics of the F1 plants.

Results: Leaf pubescence (SCC= - 0.530), upper leaf surface (SCC= 0.473) and length of mature leaf petiole denoted significant associations with BB disease index ($P<0.05$). Threshold values of the developed model to screen vulnerability of tea plant to blister blight were 1.5 for both pubescence of tea leaves and upper leaf surface.

Conclusions: Proposed leaf morphology-based thresholds can be successfully applied for preliminary screening of BB susceptibility, prior to further confirmation with more advanced identification techniques.

Keywords: Disease Control, Fungal Infection, Marker Assisted Tea Breeding, Non-Alcoholic Beverages, Plant Inherent Resistance

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INTRODUCTION

Tea is one of the most popular non-alcoholic caffeine containing beverages in the world with high amounts of flavonoids and related bioactive compounds [1-2]. Though it remains unclear, it is considered that the tea plant (*Camellia sinensis* L.) was originated from China and spread through the other South East Asian countries as a commercial cultivation [3]. Succulent plant leaves of tea are processed to make three most popular types of tea, green (unfermented), black (fully fermented) and oolong (semi-fermented) [4].

In tea plants, a considerable crop loss results due to nutrient deficiencies, stresses from climatic variations, pests and pathogen attacks. Among diseases, Blister Blight (BB) leaf disease, is caused by the obligatory biotrophic pathogen, *Exobasidium vexans* Massee, which infects only young harvestable succulent leaves, stems and the pericarp of fruits at young stage [5]. The pathogen is spread by windborne basidiospores and infection mostly proceeds through stomata [6]. Mycelium grows intercellularly prior to the formation of basidia fruiting bodies on epidermis. When they grow further, initially small pinhole size spots become visible on young leaves. As the leaves develop the spots become transparent, larger and light brown in colour. They force up and rupture the lower epidermis to form blisters with dark green and water-soaked zones surrounding the blisters [7]. After releasing the fungal spores, the blisters become velvet and white and which subsequently turns into brown in colour [8].

The BB disease causes approximately 25% - 30% crop loss per annum in Sri Lanka. Infected harvestable leaves directly reduce crop yield, not only quantitatively but qualitatively as well, due to the changes of the composition of leaf biochemical compounds such as polyphenols, catechins, enzymes etc. [9]. As the environmental conditions such as high humidity and limited sunshine directly fascilitate the infection and development of BB [10] in the field, it is important to control these microclimatic factors, in which BB may develop.

Morphological characteristics of the infected plants can be used to develop a clear relationship between the morphological characteristics of plants and blister blight resistance using an accurate scoring system for BB susceptibility of plants. Subsequently Quantitative Trait Locus (QTL) mapping can be done for proper identification of the infected plants. Evaluating the agricultural significance of fungal leaf diseases and developing tools that enable rapid recognition of diseases are very important to eliminate these pathogens [11-12].

Various methods are used to identify different pathogens, which cause diseases in crops. Morphological and genetic analysis of infected plants are two main approaches to studyplant pathogen interactions and disease development. Ponmurugan and Baby [13] conducted a study on the morphological, physiological, and biochemical changes in tea plants due to Phomopsis infection. Physiological parameters; photosynthetic and transpiration rates, stomatal conductance, efficiency of water usage and total chlorophyll content were scored both in susceptible TRI-2024 and tolerant TRI-2025 tea cultivars. Plant height, dry weight and plant strength were recorded as morphological characteristics, while total sugar, nitrogen, amino acids, protein, polyphenols and catechins of infected and healthy plants were studied as biochemical parameters. Results revealed all the morphological, physiological and biochemical characteristics tend remain significantly low in infected plants, compared to healthy plants [13].

Growth, photosynthetic and biochemical responses of tea cultivars to BB infection has been studied by Premkumar *et al.* [14], where susceptibility to BB infection has denoted significant strong associations with physical barriers, physiological and biochemical parameters (leaf area, shoot length and moisture contents etc). Not only the characteristics of the plants, but also the variations in infection causing pathogens (*Exobasidium vexans* Massee) have been studied by Abeysinghe *et al.* [15] using infected tea leaf samples. As the infection has a short (11-28 days), but multiple disease cycles with several generations within a single crop season, it requires repeated applications of fungicides to control the disease [6]. However, continuous application of fungicides can contribute to the development of new strains of *Exobasidium vexans* Massee. Morphological parameters of the pathogen such as colour, length and width of spores and DNA finger printing analysis using RAPD have revealed a high degree of genetic diversity among the samples of the *E. vexans* as an adaptation due to various conditions [16].

Plants utilize structural and chemical characteristics to prevent or reduce the spread of pathogens, which act as their first line of defence against pathogens [17]. Development of plant inherent resistance of tea cultivars against BB is the most suitable solution to control the disease, instead of applying highly toxic fungicides. In this process, marker (morphological, biochemical and molecular) assisted tea breeding is playing a key role. Among those, biochemical markers and molecular markers are more accurate and precise techniques [18-19]. However, the cost and technical requirements of molecular markers and biochemical markers are very high and the process is time consuming. Therefore, developing a simple and rapid assessment approach for early detection of BB resistance is immensely important. Hence, this study aimed to develop an inexpensive, user friendly, accurate and reliable morphological marker for preliminary screening of BB resistance traits in tea cultivars.

METHODOLOGY

Materials

In the present study, 300 individuals of an F_1 segregating population derived from a cross between TRI 2043 (a tea cultivar resistant to BB disease) and TRI 2023 (a cultivar susceptible to BB) were used in each replicate. Three replicates were grown following Randomized Complete Block Design (RCBD) together with their parents at St. Coombs Estate (Up country wet zone of Sri Lanka), Tea Research Institute, Talawakelle, Sri Lanka.

Assessment of Blister Blight Disease Severity of the F_1 Individuals

Blister Blight Disease Index (BBDI) of each F_1 individual was calculated using the data collected from the field assessments starting from year 2007 to 2010 at one-week intervals based on guidelines given in the BB severity assessment key [19].

Morphological Characterization of F_1 Individuals

Morphological assessment of F_1 individuals was carried out using the descriptors for *Camellia sinensis* L. described by the International Plant Genetic Resources Institute (IPGRI) [20]. Shape of the 5th leaf, size of the 5th leaf, leaf color, apex shape of the 5th leaf, habit of the 5th leaf apex, shape of the 5th leaf base, pubescence of the 1st leaf, leaf venation, leaf vestiture, upper leaf surface, length of the 5th leaf, width of the 5th leaf, length of mature leaf petiole, leaf length to width ratio were the fourteen morphological characteristics scored in this study. Non parametric data were converted into numerical values on the scale mentioned by the IPGRI [20]. The assessment was repeated for each individual established in three different locations and the average of each parameter was used for the statistical analysis.

Statistical Analysis

Offspring of the studied population were separated into two sets of samples based on the seed bearer (mother). The offspring produced from the seeds of TRI 2043 was considered as group 1, while group 2 consisted of the offerings from the seeds of TRI 2023. Spearman's correlation analysis was used to assess the association between different morphological characteristics of the plant and the BBDI. Further, data obtained from morphological characteristics were subjected to regression modelling, after square root transformation in order to develop a model on susceptibility to BB. In addition, Receiver Operating Characteristic (ROC) analysis was used to define the risk thresholds for susceptibility to BB based on the significantly associated morphological characteristics of the F_1 plants.

RESULTS AND DISCUSSION

Blister Blight Disease Severity of F1 Individuals

Individuals with high BB resistivity were grouped on the left-hand side of Figure 1, while high BB susceptible individuals were grouped at the right-hand side. According to the BB severity assessment key, individuals with less than 0.1 BBDI were considered as high BB resistant and individuals with a BBDI higher than 0.5 were considered as highly BB susceptible individuals. According to the analysed data, P219, P58 and P1040 were extremely resistant F_1 individuals. Meanwhile, P219, P1016 and P1018 were extremely susceptible F_1 individuals.

Morphological Characterization of F1 Individuals

The morphological characteristics of all 300 F_1 individuals were assessed and the results of six F_1 individuals from two extremes with parents are given in Table 1.

Impact of Mother Plant on the Morphological Characteristics and Incidence of Blister Blight of the Offspring

Among the notable variations in the morphological characteristics of the two offspring groups, only eight morphological characteristics; leaf colour, leaf apex shape, leaf apex habit, leaf vestiture, upper leaf surface, length of mature leaf, width of mature leaf and length of mature leaf petiole, advocated significant differences at 95% level of significance, in accordance with the statistics of the t-test (Table 2). However, the BBDI values of the two test groups did not show any significant variations (P<0.05).

Impact of Leaf Morphology on the Incidence of Blister Blight

Among the studied leaf morphological characteristics, leaf shape, leaf apex habit, leaf pubescence, leaf vestiture and leaf length to width ratio denoted negative relationships with the BBDI, while rest of the characteristics indicated positive associations. However, only the associations of leaf pubescence, upper leaf surface and length of mature leaf petiole were significant at 95% level of confidence (P<0.05), in accordance with the Spearman's correlation analysis (Table 3).

Leaf pubescence advocated a significant negative moderate relationship with the BBDI (Spearman's Correlation Coefficient [SCC]=- 0.530), while on the other hand, a significant positive moderate association (SCC=0.473) was indicated by the upper leaf surface. Regardless of the significance in correlation, the impact of leaf length to width ratio on BBDI remained to be poor (SCC<0.1).

Morphological Characteristics which Affect the Susceptibility to Blister Blight

The regression analysis based on backward elimination, yielded a simple model for the identification of susceptibility of a tea plant based on the morphological characteristics. As indicated by the model, the susceptibility of the plant remains as a function of Upper Leaf Surface (ULS) and Leaf Pubescence (LP), as indicated by the Equation 1. The model was characterized by a R^2 value of 0.57, followed by an adjusted R^2 value of 0.51.

$$Succeptibility\ to\ Blister\ Blight\ Disease = 0.78 + (0.60\ X\ ULS) - (0.25\ X\ LP) \quad (1)$$

Definition of Risk Thresholds for Blister Blight based on Leaf Morphology

The Receiver Operating Characteristic (ROC) curve analysis yielded an area coverage of 0.534 and 0.479, for upper leaf surface and leaf pubescence, respectively, while the incidence of BB was defined as BBDI>0.1 (Figure 2).

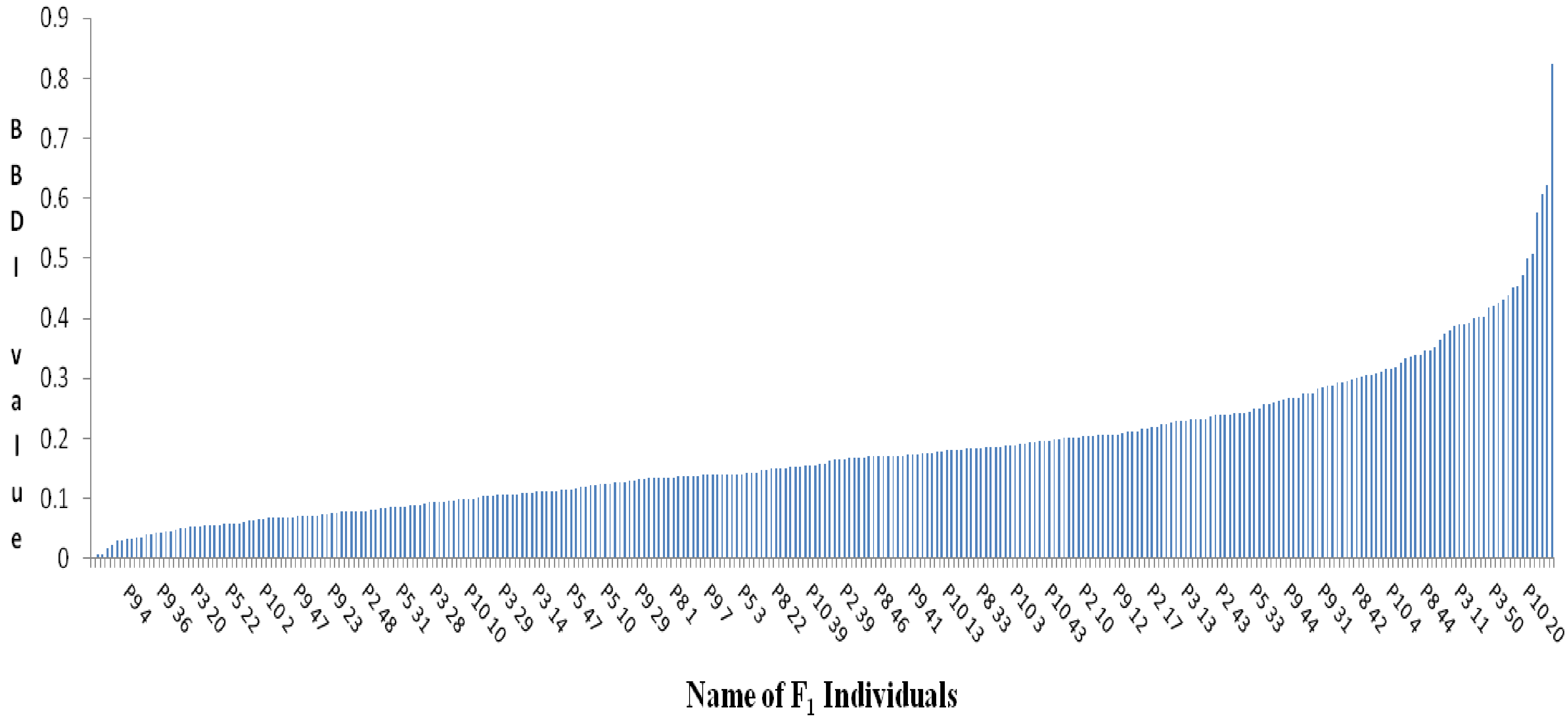

Figure 1: The Bar Chart of 300 F1 Individuals against Blister Blight Disease Index

Table 1: Morphological Characteristics of Selected Six F_1 Individuals from the Two Extremes (Highly Resistant and Highly Susceptible) of the BBDI, along with Their Parents

Sam.	Leaf shape	Leaf Size	Leaf Colour	Leaf Apex Shape	Leaf Apex Habit	Leaf Base Shape	Leaf Pubescence	Leaf venation	Leaf Vestiture	Leaf Upper Surface	Leaf Length (cm)	Leaf Width (cm)	Length of Leaf Petiole (cm)	Leaf Length Width Ratio
$P_5$8	Lanceolate (4)	Oblong (2)	Greyed yellow (4)	Acute (1)	Down turned (1)	Rounded (2)	Intermediate (5)	Distinct with bullations (2)	Pubescent (3)	Rugose (2)	8.00	3.70	0.36	2.15
$P_9$20	Lanceolate (4)	Oblong (2)	Greyed green (3)	Acute (1)	Down turned (1)	Rounded (2)	Intermediate (5)	Distinct with bullations (2)	Pubescent (3)	Rugose (2)	8.30	3.10	0.33	2.70
P_{10}40	Lanceolate (4)	Oblong (2)	Greyed yellow (4)	Acute (1)	Down turned (1)	Attenuate (1)	Sparse (3)	Distinct with bullations (2)	Pubescent (3)	Smooth (1)	8.80	3.60	0.40	2.44
$P_2$19	Lanceolate (4)	Oblong (2)	Greyed yellow (4)	Acute (1)	Down turned (1)	Attenuate (1)	Intermediate (5)	Distinct with bullations (2)	Pubescent (3)	Smooth (1)	8.30	3.60	0.38	2.31
P_{10}16	Lanceolate (4)	Oblong (2)	Green (2)	Acute (1)	Down turned (1)	Attenuate (1)	Sparse (3)	Distinct with bullations (2)	Pubescent (3)	Rugose (2)	9.90	4.50	0.66	2.20
P_{10}18	Lanceolate (4)	Oblong (2)	Green (2)	Acute (1)	Down turned (1)	Attenuate (1)	Sparse (3)	Distinct with bullations (2)	Pubescent (3)	Smooth (1)	6.50	3.10	0.35	2.06
TRI 2023	Lanceolate (4)	Oblong (2)	Yellow green (5)	Acute (1)	Down turned (1)	Attenuate (1)	Sparse (3)	Distinct with bullations (2)	Pubescent (3)	Smooth (1)	11.80	4.70	0.44	2.52
TRI 2043	Lanceolate (4)	Oblong (2)	Greyed yellow (4)	Acute (1)	Down turned (1)	Attenuate (1)	Dense (7)	Distinct with bullations (2)	Pubescent (3)	Rugose (2)	10.20	4.30	0.45	2.38

***Note:** Sam.: Sample*

Table 2: Results of the t-Test for Significant Differences among Leaf Morphological Characteristics between the Two Groups (Highly Resistant and Highly Susceptible) of Off springs

Morphological Parameters	Mean Value		F Value	t Value	df	p Value
	Group 1	Group 2				
Leaf shape	3.6	3.8	12.756	1.749	298	0.081
Leaf colour	1.15	1.30	18.983	-2.124	298	0.034*
Leaf apex shape	2.30	2.81	146.777	-5.390	298	0.001*
Leaf apex habit	1.24	1.14	21.210	2.247	298	0.025*
Leaf base habit	1.18	1.13	5.097	1.053	298	0.293
Leaf pubescence	2.54	2.66	0.561	-1.621	298	0.106
Leaf venation	2.00	2.00	0.426	-0.773	298	0.440
Leaf vestiture	4.43	4.57	68.506	3.787	298	0.001*
Upper leaf surface	1.96	1.83	0.484	-4.986	298	0.001*
Length of mature leaf	10.48	11.69	0.669	-3.705	298	0.001*
Width of mature leaf	4.38	4.71	2.663	2.210	298	0.028*
Length of mature leaf petiole	0.71	0.68	0.117	-3.131	298	0.002*
Blister Blight Severity Index	0.2	0.19	0.629	1.071	298	0.285

Note: *"*" in the column indicates significant difference (P<0.05) among the two groups in accordance with the t-test*

Table 3: Results of the Correlation Analysis between Different Leaf Morphological Characteristics and Blister Blight Disease Index

Morphological Parameter	Spearman Correlation Coefficient (SCC)
Leaf shape	-0.004
Leaf colour	0.026
Leaf apex shape	0.083
Leaf apex habit	-0.053
Leaf base habit	0.047
Leaf pubescence	-0.530*
Leaf venation	0.026
Leaf vestiture	-0.022
Upper leaf surface	0.473#
Length of mature leaf	0.026
width of mature leaf	0.066
Length of mature leaf petiole	0.131*
Leaf length to width ratio	-0.036

Note: *"*" denotes parameters that indicated a significant correlation with BBDI (P<0.05) at 5% level of significance, while "#" denotes parameters significant at 1% level of significance (P<0.01).*

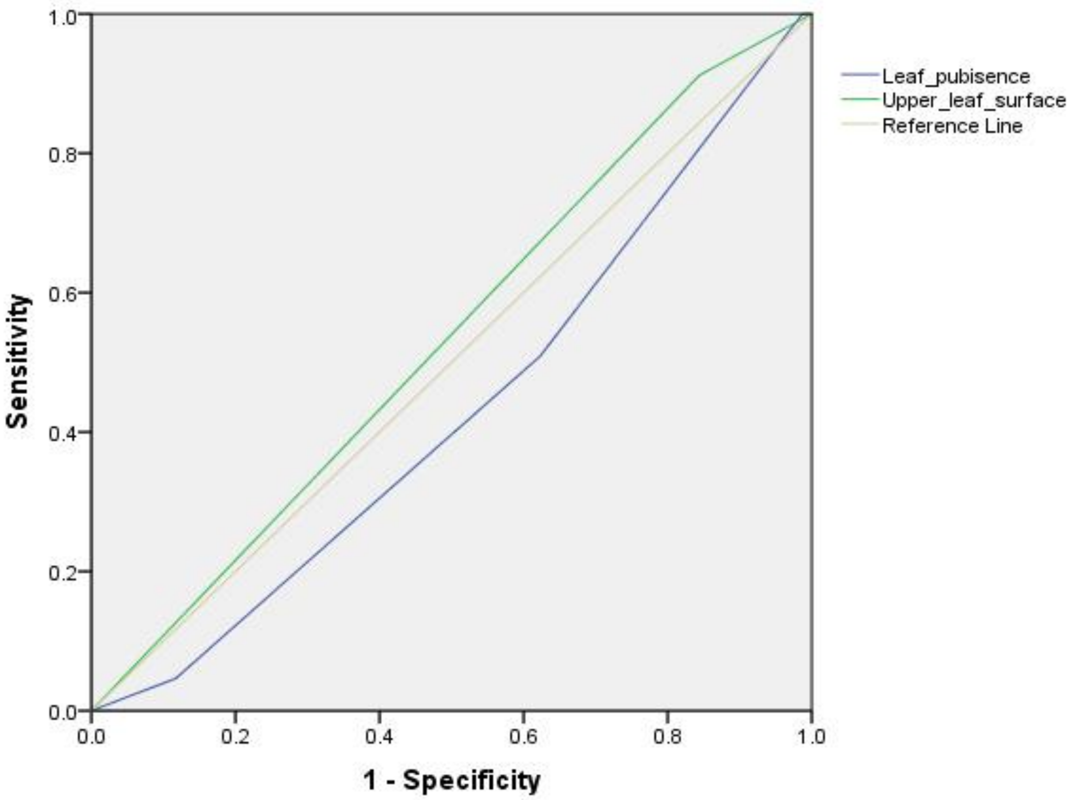

Figure 2: ROC Curve for the Leaf Pubescence and Leaf Vestiture Associated with BBDI

Therefore, it was reassured that both of these leaf characteristics are significantly associated with the incidence of BB. Based on the distribution of the curve and the coordinates of the curve (Figure 2) (sensitivity and 1– specificity), leaf pubescence>1.5 and upper leaf surface>1.5, categories could be considered as risk thresholds, which can symbolize the susceptibility of tea plants to BB incidence. In the analysis of the BB severity of both F_1 individuals derived from TRI 2043 and TRI 2023, resistance and susceptibility characteristics have not shown any maternal segregation as denoted by the statistics of the t test.

As suggested by the overall results of Spearman's correlation analysis, morphological characteristics such as leaf shape, leaf apex habit, leaf pubescence, leaf vestiture and leaf length to width ratio have denoted negative relationships with BBDI. Narrow leaf with notable length has decreased the susceptibility to the BB. The ratio between leaf length and leaf width also have shown negative correlations with BBDI. Therefore, lanceolate shaped leaf with higher value of ratio between leaf lengths to width can be considered as more resistant to blister blight disease, than ovate, oblong and elliptic leaf shapes. Lanceolate shaped leaves are more resistant to BB, due to the availability of a narrow space for accumulation of BB spores.

In the current study, leaf pubescence has also indicated a significant negative correlation with the BBDI. The leaf pubescence was observed under microscope and categorized as; sparse, intermediate or dense. When pubescence density increases, it can act as a physical barrier for infection [21] limiting the susceptibility to BB. In a study conducted for Uromyces, the presence of dense leaf pubescence has been documented to retard the germination of spores on the surface of bean leaves by trapping the spores [22], thereby reducing the probability of germ tubes reaching the penetration site [24]. A high density of trichomes can also prevent mycelial penetration and infection of other biotrophic fungi [24]. It is reported that an increased number of hydrophobic pubescence may repel water from the leaf surfaces, thus preventing successful penetration of fungal germ tubes [24]. Alternatively, a high trichome number may simply reduce the frequency of germ tube contact points that can lead to penetration [25]. The straight leaf apex habit was more vulnerable to disease infection rather than down turned leaf, as suggested by the negative correlation between leaf apex habit and BBDI.

On the other hand, several factors such as leaf colour, leaf apex shape, leaf base habit, leaf venation, upper leaf surface, length of mature leaf, length of mature leaf petiole have shown positive correlations with BBDI. All the positively correlated characteristics increase the probability of being infected by pathogen spores, through facilitating the trapping of spores and providing more surface area to interact with the plant leaf. Increase of the leaf length and leaf width may increase the surface area of the leaf and allow the spores of pathogens to increase the chance of contamination [12].

Distinct mid rib and lateral leaf venation system with bullate has also made the leaf more vulnerable to disease infection, than indistinct sunken leaf venation in lamina. It may facilitate the trapping of spores in the wind. When considering the impact of leaf base area, the susceptibility to BB tend to increase from attenuate to blunt shapes, when the leaf base surface area increases. Leaf apex shape denoted a positive correlation with BBDI and therefore the BB severity tend to vary as acute < obtuse < attenuates in shapes, respectively.

Most outstanding leaf morphological characteristics such as upper leaf surface and length of mature leaf petiole were denoting significant positive correlations with BBDI ($P<0.05$ at 95% level of confidence). A rough upper leaf surface generally leads to high retention of fungal spores, while increasing the length of leaf petiole, may favour the exposure to spores of pathogen [26].

After considering the correlation of all the studied leaf morphological parameters with susceptibility to BB, a simple model to predict the vulnerability of a tea plant to BB (based on morphological features) was developed through step-wise regression analysis. However, the current model only considers the leaf morphological factors with less attention on other external environmental factors such as soil nutrients, light etc. Further, regardless of the combined effect of upper leaf surface and leaf pubescence in terms of BB susceptibility, individual thresholds for each parameter were also developed through a ROC analysis. However, it should be noted that both, upper leaf surface and leaf pubescence are non-parametric morphological parameters and the model was derived with a limited number of samples. Therefore, the current thresholds and leaf morphology-based model is recommended for preliminary screening of BB susceptibility, due to its rapid and limited resource consumptive (labour and cost) nature, prior to further confirmation with more precise molecular markers.

CONCLUSIONS

Constitutive barriers limited or completely inhibited the penetration of tea tissues by pathogenic fungi. The resistant individuals of the analyzed F_1 segregation population were characterized by a significantly higher pubesence density, than susceptible forms. In resistant individuals, upper leaf suface was smooth, which minimise the accumilation of pathogen spores.

Based on the findings, upper leaf surface and leaf pubescence can be used to evaluate the susceptibility to BB incidence in tea plants. The proposed model can be used for preliminary evaluation of BB resistant or BB succeptible traits and it should be validated with more tea cultivars in different ecological regions to enhance the reliability and accuracy.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

TK: Carried out the investigations, data collection, supported the statistical analysis, and wrote the manuscript; MK and JW: Supervised the study; LU: Analysed data and wrote the manuscript; CP: supervised the study and revised the manuscript; NE: Supported data collection process. All authors read and approved the manuscript.

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APPLIED BIO-SYSTEMS TECHNOLOGY

Research Article **Open Access**

Efficacy of Liquid Organic Fertilizers Derived from *Eichhornia crassipes*, *Tithonia diversifolia* and *Gliricidia sepium* on the Growth of *Ipomoea aquatica* under Hydroponic Conditions

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Abstract

Background: The current study was conducted to evaluate the efficacy of liquid organic fertilizers produced from the extracts of three plant species, namely, *Eichhornia crassipes*, *Tithonia diversifolia* and *Gliricidia sepium* on the growth of *Ipomoea aquatica* under hydroponic conditions.

Methods: Six liquid organic fertilizer treatments were prepared form the aforementioned plant extracts and were used to cultivate *Ipomoea aquatica*, under hydroponic settings. Each treatment consisted of ten plants and the control system contained Albert solution. The prepared hydroponic systems were arranged in a Completely Randomized Design inside a semi protected plant house and the growth parameters of the plants were recorded up to 60 days. The General Linear Model (GLM) was used for the statistical comparisons.

Results: All the parameters denoted significant differences among the treatments ($P<0.05$), except for dry root weight, plant height and chlorophyll content. The Treatment 2 denoted the highest mean values for the vegetative parameters including, root length (18.2 ± 2.4), fresh root weight (0.44 ± 0.02), dry root weight 0.05 ± 0.01), dry shoot weight (0.21 ± 0.01), number of leaves (8.7 ± 0.6) and plant height (39.5 ± 3.3), while reporting the second highest values for fresh shoot weight (1.57 ± 0.1) and leaf area (48.1 ± 9.8).

Conclusions: Based on the findings, T2 treatment (*Eichhornia crassipes* 50% + Water 50%) can be recommended as the best performing liquid organic fertilizer medium, to be used in hydroponic cultivation systems.

Keywords: *Eichhornia crassipes, Gliricidia sepiumv,* Hydroponic Systems, Organic Fertilizers, *Tithonia diversifolia*

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INTRODUCTION

With the increasing population growth and climate change, ensuring the food security has become a serious concern at both national and global scales. Hence the need for higher productivity with the efficient use of inputs has become a constantly increasing challenge in modern agriculture [1]. Meanwhile, development of the agricultural sector has increased the detrimental impacts on natural ecosystems due to bio-accumulation and bio-magnification of agrochemicals, especially due to the intensive use of synthetic pesticides and fertilizers. Regardless of the efficacy attained from intensive crop production systems, accumulation of chemical residues, leading to different health impairments has become a serious concern [2]. With respect to fertilizers, one of the highest direct form of expenses in farming, the over usage of synthetic chemical pesticides has been a serious issue, which has caused severe negative impacts on the environment and human health. In addition, excessive use of inorganic fertilizers has also created a number of environmental problems, with the most serious one being the built up of phosphate and nitrogen compounds in water and the atmosphere [3].

Therefore, fertilizer application must be practiced in an appropriate manner to maintain the sustainability of the ecosystems, while balancing the cost effectiveness and convenience. However, with the realization of the adverse impacts of prolong and excessive use of agro-chemicals, the demand for adopting eco-friendly agricultural practices for sustainable food production has increased [4]. This has emphasized the necessity of searching for novel fertilizer, herbicide, pesticide and weedicide formulations, which are efficient, effective and environmentally friendly [5, 6].

Organic fertilizers are considered as an effective mode of promoting environmental sustainability, while sustaining the soil fertility and plant growth in the long run [7, 8]. Among different organic fertilizers, application of liquid organic fertilizers is a widely adopted strategy in modern crop management, due to the high nutrient-use efficiency. The source and the physical nature of the fertilizer is having a significant effect on the performance of plants. In this context, liquid fertilizers are convenient and effective method to enhance the nutrient availability, due to the presence of water that ensures uniformness in nutrient mixing [9]. Liquid organic fertilizers consist of essential plant nutrients and beneficial micro-organisms, which are recycled organic matter, formulated from natural materials of either plant or animal origin [10, 11]. Compost extracts, aerated compost teas, herbal extracts, vermicompost extracts and food stillages are few widely utilized organic liquid fertilizers [12-14].

Hydroponics or soil-less culture is a technology for growing plants in mineral nutrient solutions, where nutrients are fed directly to the roots, along with elements needed for optimum plant growth with or without the use of an inert medium such as gravel, rock wool, peat moss, saw dust, coir dust or coconut fiber [15, 16]. This method is a sustainable alternative to constraining factors in conventional agriculture, offering better nutrient control, less labour, relatively less cost and time requirements for land preparation, while being free from soil borne pathogens. The plant density per unit area of hydroponic systems can be doubled, so that it enhances the productivity, while providing with quality products. According to literature, hydroponic technology can reduce land requirements for crops by 75% or more, and water use by 90%. Further this enables continuous cultivation cycles, regardless of certain serious limitations in soil and environmental concerns [17].

Apart from using this technology in commercial scale cultivation projects, hydroponic cultivation system is a better counterpart in urban agriculture, which enables urban crowd in obtaining their own harvest of crops within the limited land availability. Hence, this can improve the living spaces physically and psychologically, while ensuring ecological sustainability of urban landscapes [18].

Even though, hydroponics is an excellent technique for the cultivation of vegetable crops and other plants, it often utilizes inorganic fertilizers [4]. In recent years, peoples' consciousness on food-safety and environment has increased, which have lifted the interest in organic farming techniques. Hence, hydroponic producers are facing a challenge of adopting in to "organic hydroponic systems", by developing a satisfactory organic nutrient medium. However, a limited number of studies have focused on development of an organic fertilizer medium to be used in hydroponic systems, while ensuring a satisfactory productivity. Therefore, this study attempted to develop an organic nutrient medium produced from the extracts of three plant species, namely *Eichhornia crassipes* (EC), *Tithonia diversifolia* (TD) and *Gliricidia sepium* (GS).

METHODOLOGY

Experiment Site

The study was conducted at the Faculty of Agriculture and Plantation Management Wayamba University of Sri Lanka, Makandura located in the low country intermediate zone (IL1a).

Preparation of Extracts

Three plant species, namely Water Hyacinth (*Eichhornia crassipes*), Wild/Mexican Sunflower (*Tithonia diversifolia*) and Gliricidia (*Gliricidia sepium*) were considered for the development of an organic liquid fertilizer, as shown in Figure 1. *Eichhornia crassipes* (Water Hyacinth) is a floating aquatic plant that belongs to the family Pontederiaceae (Figure 1). It is native to South America, and the rapid growth and reproduction of water hyacinth has made it to be distributed throughout the world. This plant is considered as an invasive plant, which adversely affects the quality and functionality of freshwater bodies in Sri Lanka [19]. *Tithonia diversifolia*, (Mexican Sunflower) belongs to the family Asteraceae and it is widely distributed throughout the South America, Asia and Africa. Mexican sunflower is used for a variety of purposes including, ornamental, as a fuel, for compost preparation, land demarcation, soil erosion control, soil remediation, as building materials and shelter for poultry etc. This plant is having a high potential as a green manure. The green biomass of *Tithonia diversifolia* is an important resource of nutrients, which contains notable amounts of Nitrogen (3.5%), Phosphorous (0.37%) and Potassium (4.1%) [20].

Gliricidia sepium, (Gliricidia) is a leguminous tree belonging to the family Fabaceae (Figure 1). It has spread from its native range throughout the tropics due to its diversified uses in crop management aspects. It serves as shade tree in plantation crops, while being used for green manure, fodder, live fencing, intercropping etc. The high initial Nitrogen, low Carbon Nitrogen ratio (C: N; lignin+ polyphenol), generally favour high rates of decomposition of fresh leguminous leaves, making Gliricidia a good candidate for development of a nutrient medium [21].

The plant materials were collected from the Kurunegala District. The collected plant materials were dried in room temperature for 4-5 days to precondition the extraction. The plant extractions were carried out according to the standard procedures recommended by Andika *et al*. [22] and Kolhe and Singh [23]. At the end of digestion period, the individual extracts were obtained, separately. The extracts were subsequently mixed in different ratios to prepare six treatments, as shown in Table 1. A recommended dosage of Albert solution was used as the control.

Experimental Design

The efficacy of the prepared plant extract formulations was evaluated using *Ipomoea aquatica* (Kangkung), a popular leafy vegetable crop in Sri Lanka. The crop was established under non circulating hydroponic settings (trough culture) using commercially available seeds. A total of ten *Ipomoea aquatica* plants were introduced to each fertilizer treatment, which were arranged in a Completely Randomized Design (CRD) with three replicates for each, inside a semi protected plant house. The plants were maintained for five weeks, since transplanting.

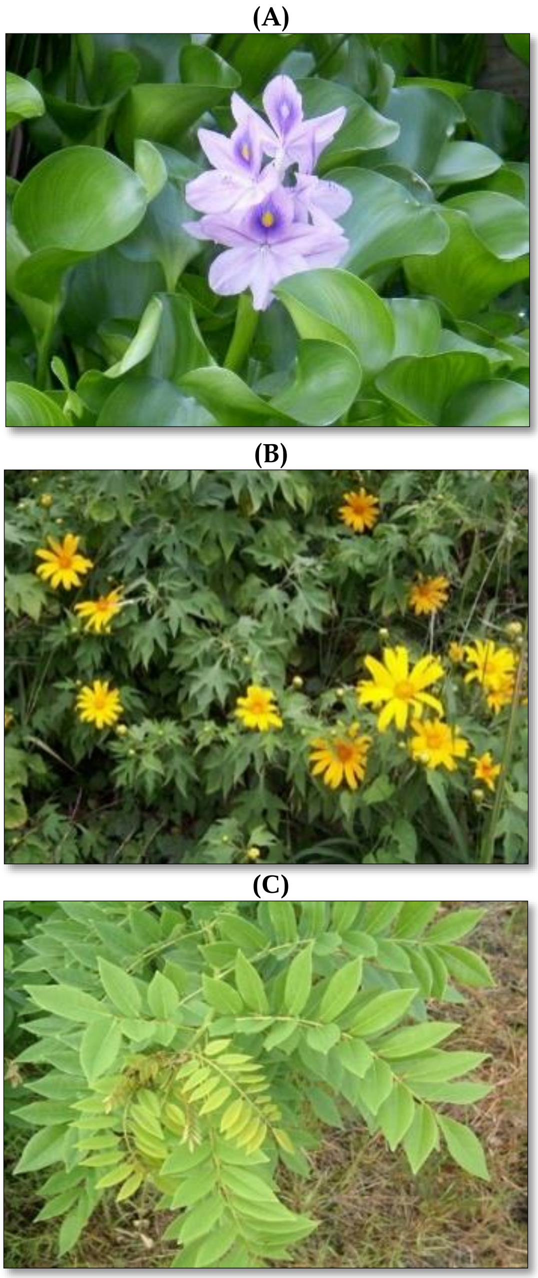

Figure 1: (A): Water Hyacinth; (B): Wild/Mexican Sunflower and (C): Gliricidia plants

Table 1: Treatments used in the Study

Code	Treatment
T1	Albert solution (recommended dosage) (control)
T2	EC 50% + Water 50%
T3	EC 25% + Water 75%
T4	EC 25% + (TD + GS Mixture)25% + Water 50%
T5	EC 25% + (TD + GS Mixture)50% + Water 25%
T6	TD + GS Mixture)50% + Water 50%
T7	TD + GS Mixture)75% + Water 25%

Note: EC: *Eichhornia crassipes;* TD: *Tithonia diversifolia;* GS: *Gliricidia sepium*

Data Recording and Analysis

Selected vegetative parameters were recorded from all plants in each treatment. Plant height (cm) were measured from the base of the plants, along with the number of leaves per plant. Further, the Chlorophyll contents were recorded using a SPAD Meter (S 502 plus) at weekly intervals starting from the first week. In addition, the total leaf areas (cm^2) of the plants were measured using a bench top leaf area meter (Li-3100C) at weekly intervals.

The root length (cm) and fresh weight of above ground biomass (g) were measured after five weeks, since transplanting. The oven drying method at 80 °C for 48 h was used to determine the dry weight of the above ground biomass (g). Further pH and Electrical Conductivity (EC) levels of every treatment were monitored weekly using a pH meter and an Electrical conductivity meter. The nutrient analysis was done to identify the Nitrogen, (Kjeldahl method), Phosphorus (Olsen method) and Potassium (Flame Photometer), composition of each plant extract. The recorded data was analyzed using the General Linear Model (GLM) followed by the Tukey's pair-wise comparison for mean separation. All statistical analysis were performed in SPSS (version 23).

RESULTS AND DISCUSSION

Nutrient Analysis

According to the initial nutrient analysis of the stock solutions, the highest N level (0.075 %) was found in gliricidia extract, while the highest P (70.43 ppm) and K values (1076.0 ppm) were recorded by the extract of giant Mexican sunflower (Table 2).

Variations in Vegetative Parameters

Nutrient element combinations, concentration and adequate supply heavily influence the plant growth and development. Hence, plant growth parameters can be directly influenced by the fertilizers. The studied morphological and physiological parameters of the *Ipomoea aquatica* plants revealed different responses to varying treatments of fertilizers.

Plant Height

Plant height is an important morphological phenotype, which is a direct identifier of the overall plant growth. In this study, plant height did not indicate any significant variations among the treatments (P>0.05 at 95 % level of confidence), as shown in Table 3. However, the highest mean value for plant height was observed under T2 (39.5 ± 3.3 cm), while the lowest value was observed from T3 treatment (27.0 ± 1.8 cm). A similar study by Andika *et al.* [22], which has been conducted to evaluate the effect of a water hyacinth liquid fertilizer on *Crotalaria ochroleuca*, has denoted a significant proliferation of plant height over the inorganic liquid fertilizers. Further Abu, [24] has reported that an organic fertilizer developed with water hyacinth, has resulted a significant effect on the plant height of *Colocasia esculenta*. Additionally, organic fertilizer extracts from Tithonia and Gliricidia and cocao skin, have also shown promising results on plant growth, indicating a significant increase in plant height of water spinach, lettuce and cauliflower like crops [6, 25].

Number of Leaves and Total Leaf Area

Number of leaves and leaf area determines the light interception capacity of a crop and is often used as a key plant growth parameter, which will influence the photosynthetic rate and carbon partitioning [26]. As per the results of the current study, the leaf counts denoted significant variations among different treatments (P<0.05 at 95% level of confidence). The highest mean value for number of leaves was observed in T2 as 8.7 ± 0.6 leaves, followed by T7 (6 ± 1.6 leaves), while T3 reported the lowest value as 4.0 ± 0.4 leaves, as shown in Table 3.

Similarly, the total leaf area also denoted significant variations among the treatments (P<0.05). According to the results, the highest mean value for total leaf area was observed under T1 (48.4 ± 6.1 cm^2), while the lowest value was observed from T3 treatment (14.0 ± 2.0 cm^2). A similar trend was observed during a previous study by Talkah [27], while using organic fertilizer extracts for plant taro (*Colocasia esculenta* L.).

Further, several other experiments on organic hydroponic cultivation of crops have also denoted higher leaf numbers and leaf area with the treatments of plant extracts compared to the inorganic control treatments [25, 28-29].

Table 2: Nutrient Composition of the Pure Extracts

Plant Material	Nitrogen (N) %	Phosphorous (P) ppm	Potassium (K) ppm	pH value	EC Value (mS/cm)
Water Hyacinth	0.075	14.75	902.20	7.2	4.38
Giant Mexican Sunflower	0.016	70.43	1076.00	6.9	7.74
Gliricidia	0.109	63.75	712.0	5.6	7.57

Chlorophyll Content

Chlorophyll is an essential element for photosynthesis, where high chlorophyll content enables efficient gain of energy and production of foods [30]. Hence, chlorophyll content acts as an important indicator of plant health. Despite being non-significant, the highest mean value for chlorophyll content was observed in T1 as (36.22 ± 1.3 SPAD Units [SU]), followed by T4 (34.63 ± 2.6 SU), while T3 reported the lowest value (28.87 ± 3.2 SU), as shown in Figure 2. As previously stated, no significant variation in chlorophyll content was observed among these seven treatments. (P>0.05 at 95 % level of confidence). Organic fertilizers are good sources of nitrogen, which favour chlorophyll production. This scenario has been further emphasized by the previous studies conducted with different plant extracts [8, 31].

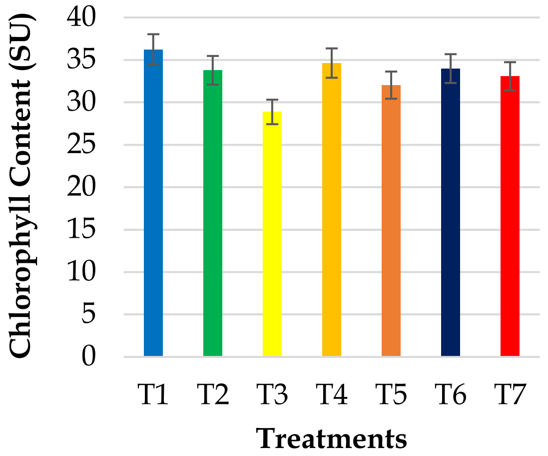

Figure 2: Chlorophyll Contents of *Ipomoea aquatica* Plants Grown under Different Treatments

Root Length

Root development is an important parameter of plant development. The root systems of plants perform important roles in plant growth by actively mediating the acquisition of nutrients and water to plants, while facilitating other functions such as anchorage, synthesis of plant hormones etc. [32]. The root development of a plant is profoundly regulated by genetic aspects, as well as external environmental factors such as nutrient levels [33].

According to the results of the current study, the root length denoted a significant variation among the treatments (P<0.05 at 95% level of confidence) as shown in Table 3. The highest mean value for root length was reported under T2 (18.2 ± 2.4 cm), while the lowest value was reported from T3 treatment (7.2 ± 0.9 cm). A study conducted by Ji *et al.* [8], has reported an effective root development in chrysanthemum with the liquid organic fertilizer treatments that they have tested, while Andrian *et al.* [28] and Phibunwatthanawong and Riddech [11] have further experienced similar observations in root length with the organic fertilizer treatments in hydroponically grown water spinach and lettuce, respectively.

Fresh Weight and Dry Weight

Different sources of nutrient (organic or mineral) possess a significant effect on total plant biomass. When the fresh weight is considered, it can be separately measured as fresh shoot weight and fresh root weight. Both of these parameters were indicating significant variations among the treatments (P<0.05 at 95% level of confidence), as shown in Table 3. The highest mean value for fresh shoot weight was reported under T5 (1.59 ± 0.2 g), while the lowest value was reported from T3 treatment (0.75 ± 0.1 g). In case of fresh root weight, the highest mean value was observed under T2 (0.44 ± 0.02 g), while the lowest value was observed from T7 treatment (0.12 ± 0.1 g).

Same as fresh weight, dry weight was also measured separately as the dry shoot weight and dry root weight. Dry shoot weight denoted significant variations among treatments (P<0.05 at 95% level of confidence). In here, the highest mean value was observed under T2 (0.21 ± 0.01 g), while the lowest value was observed from T3 and T4 treatments (0.10 ±0.01 g). Meanwhile, the dry root weight did not indicate any significant variations among treatments (P<0.05 at 95% level of confidence). However, the highest mean value for dry root weight was observed under T2 (0.05 ± 0.01 g).

Table 3: Summarized Mean Values for Morphological Parameters of *Ipomoea aquatica* Treated under Different Fertilizer Combinations

Trt.	Plant Height (cm)	Number of Leaves	Root Length (cm)	Leaf Area (cm²)	Fresh Root Weight (g)	Fresh Shoot Weight (g)	Dry Root Weight (g)	Dry Shoot Weight (g)
T1	30.8 ± 1.1^{a}	5.2 ± 0.9^{ab}	7.8 ± 0.7 ^{a}	48.4 ± 6.1^{c}	0.22 ± 0.03^{a}	1.30 ± 0.1^{b}	0.03 ± 0.005^{a}	0.11 ± 0.01^{a}
T2	39.5 ± 3.3^{a}	8.7 ± 0.6^{b}	18.2 ± 2.4^{b}	48.1 ± 9.8^{c}	0.44 ± 0.02^{b}	1.57 ± 0.1^{b}	0.05 ± 0.01^{a}	0.21 ± 0.01^{b}
T3	27.0 ± 1.8^{a}	4.0 ± 0.4^{a}	7.2 ± 0.9^{a}	14.0 ± 2.0^{a}	0.25 ± 0.05^{a}	0.75 ± 0.1^{a}	0.04 ± 0.02^{a}	0.10 ±0.01^{a}
T4	33.1 ± 2.5^{a}	5.7 ± 0.4^{ab}	9.4 ± 2.2^{ab}	26.9 ± 5.2^{b}	0.17 ± 0.04^{a}	0.88 ± 0.2^{a}	0.02 ± 0.01^{a}	0.10 ±0.01^{a}
T5	38.5 ± 2.02^{a}	5.0 ± 0.5^{a}	11.9 ± 1.5^{b}	28.6 ± 5.8^{b}	0.29 ± 0.1^{b}	1.59 ± 0.2^{b}	0.05 ± 0.01^{a}	0.15 ± 0.01^{b}
T6	30.4 ± 4.1^{a}	5.0± 0.7^{a}	9.0 ± 2.3^{a}	30.7 ± 7.2^{b}	0.14 ± 0.03^{a}	1.16 ± 0.4^{a}	0.02 ± 0.004^{a}	0.10 ± 0.03^{a}
T7	35.1 ± 4.9^{a}	6.0 ± 1.6^{ab}	8.0 ± 3.8^{a}	26.0 ± 6.4^{b}	0.12 ± 0.1^{a}	1.19 ± 0.3^{ab}	0.05 ± 0.03^{a}	0.11 ± 0.01^{a}

Note: *Trt.: Treatment*

Meanwhile the lowest value was observed from T4 treatment (0.02 ± 0.01 g). Similar to inorganic fertilizers, organic fertilizers can also improve the plant biomass [34-35]. Biomass stimulation is a consequence of the hormone like effect of humic acids present in organic fertilizers [36]. Several previous studies conducted by Andrian *et al.* [28] and Setyowati *et al*. [6] have reported similar variations in plant fresh and dry weight. However, in certain cases, organic nitrogen may not significantly influence the biomass production of plants, where Kasim *et al*. [3] and Williams and Nelson [37] have reported relatively lower values of fresh and dry weights of the organically fertilized plants, in comparison with inorganically treated ones.

At present, resource constraints in agricultural production have become sterner than in the past. Hence, hydroponic/soil-less culture has been identified as a good solution, which provides many socio-economic benefits [16]. Furthermore, the soil-less cultivation approaches can ensure continuous supply of fresh and hygienic vegetables in sufficient quantities, especially in urban settings to facilitate urban agriculture under limited space conditions [15]. Application of liquid fertilizers is both effective and convenient in crop management, when compared to use of solid forms. Therefore, the use of organic nutrient solution based hydroponic systems can cater for the increasing demand in food supply, while resulting minimum impacts on the environment [16].

The findings of the current study revealed that liquid organic fertilizers can be successfully used as a substitute for conventional inorganic fertilizers in hydroponic cultivation systems. The most important factor of this study was the utilization of water hyacinth plant as a major component. Being an invasive plant, water hyacinth possesses negative effects on ecosystems and considered to be an irritation in control and management of inland waterbodies in many countries including Sri Lanka [22, 38]. Hence utilization of a such plant in an effective way as a nutrient source, would be immensely helpful in reduction and management of ecological and economic burdens caused by it [38].

CONCLUSIONS

Based on the overall findings, the T2 (*Eichhornia crassipes* 50% + Water 50%) treatment denoted the highest mean values for majority of vegetative parameters, including root length, fresh root weight, dry root weight, dry shoot weight, number of leaves and plant height, while denoting the second highest values for fresh shoot weight and leaf area. Therefore, based on the findings, out of all the tested treatments, T2 can be recommended as a successful organic fertilizer medium to be used in hydroponic cultivation systems. However, more research efforts are required for the optimization of the formulation of the fertilizers, quantifying the optimal fertilizer rate, efficacy against different crops and microbial responses in organic hydroponic systems.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

NS: Designed the study, supervised the experimental procedures and wrote the manuscript. MA, TS, AW and KW: Conducted the experiments, collected the data and prepared the first draft of manuscript. LU: Conceptualized the study, supervised the study, conducted the statistical analysis and wrote the manuscript. All authors read and approved the manuscript.

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