DEVELOPMENT AND CHARACTERISATION OF NUTRACEUTICAL HERBAL TEA FROM *POLYALTHIA LONGIFOLIA* LEAVES

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by

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LIST OF SYMBOLS

$	dollar
%	Per cent
°C	Degree Celsius
>	greater than
g	Gram
kg	kilogram
mg	milligram
mm	millimetre
lbs	pounds
ft	feet
m	meter
µg	microgram
mL	Milliliter
±	Plus-minus
µm	micrometre
nm	nanometre
V	Voltage
L	litter
≥	Greater than or equal
P	Statistical probability of the hypothesis
<	less than
®	Registered trademark

M	molar
mM	millimolar
µM	micromolar
nmol	nanomoles
cm^2	square centimetre

LIST OF ABBREVIATIONS

B.C	Before Christ
BCE	Before Common Era
C.E	Common Era
AD	Anno Domini
US	United States
EU	European Union
Cx43	Connexin 43
UK	United Kingdom
EU	Europe
CIS	Commonwealth of Independent States
tsp	id est (that is)
LDL	low-density lipoprotein
BID	bis in die (twice a day)
TID	ter in die (three times a day)
i.e.	id est, (that is)
etc	et cetera
CAGR	Compound annual growth rate
WHO	World Health Organization
TMP	traditional medicine practitioners
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
FTIR	Fourier-transform infrared spectroscopy

AOAC	Association of Official Analytical Chemists
pH	Potential of hydrogen
RNA	Ribonucleic acid
K_2SO_4	Potassium sulphate
$CuSO_4$	copper sulphate
H_2SO_4	sulfuric acid
NaOH	Sodium hydroxide
NHCl	ammonium chloride
H_3BO_3	Boric acid
NH_3	ammonia
AAS	Atomic Absorption Spectrometry
Pb	Lead
Cd	Cadmium
As	Arsenic
Hg	Mercury
OD	Optical Density
SPSS	Statistical Package for Social Sciences
MIR	Mid-Infrared
HMDB	Human Metabolome Database
MFE	molecular feature extraction
RT	Retention time
m/z	molecular mass /charge number
ADI	Acceptable Daily Intake
PTI	Provisional Tolerated Intake
CNS	Central Nervous System

HSV	herpes simplex virus
VV	vaccinia virus
ROS	Reactive oxygen species
$HO^{\bullet}$	hydroxyl radical
$O2^{\bullet-}$	superoxide radical
$NO^{\bullet}$	nitric oxide radical
$LOO^{\bullet}$	lipid peroxyl radical
H_2O_2	hydrogen peroxide
$1O_2$	single oxygen
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
FRAP	Ferric reducing antioxidant power
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
TPC	Total Phenolic Content
Na_2CO_3	sodium carbonate
TFC	Total Flavonoid Content
$AlCl_3$	aluminium chloride
CH_3CO_2K	potassium acetate
QE	Quercetin Equivalents
$K_2S_2O_8$	potassium persulfate
$FeCl_3$	ferric chloride
$FeSO_4$	ferrous sulphate
$FeSO_47H_2O$	sulfate heptahydrate
PCC	Pearson Correlation Coefficient
FRSA	Free Radical Scavenging Activity
AIDS	Acquired Immunodeficiency Syndrome

DNA	Deoxyribonucleic acid
Fe^{3+}	ferric ion
Fe^{2+}	ferrous ion
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
DALYs	Disability-Adjusted Life Years
USM	Universiti Sains Malaysia
INFORMM	Institute for Research in Molecular Medicine
CO_2	carbon dioxide
PBS	Phosphate-buffer saline
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal Bovine Serum
DMSO	Dimethyl sulfoxide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
IC	Inhibitory Concentration
CV	Cell Viability
SEM	Scanning Electron Microscopy
HBSS	Hanks Balanced Salt Solution
HMDS	hexamethyldisilazane
v/v	Volume per volume
w/v	Weight per volume
PE	Plating efficiency
SF	Surviving Fraction
PI	Propidium Iodide
SD	Standard Deviation

LDH	lactate dehydrogenase
XTT	2,3-bis (2-methoxy- 4-nitro-5-sulphophenyl)-5-carboxanilide-2H-tetrazolium
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
WST-1	(4-[3-4-iodophenyl]-2-(4-nitrophenyl)-2H-5-tetrazolio)- 1,3-benzene disulfonate
CCK-8	colorimetric counting kit-8
3D	three-dimensional
AVD	apoptotic volume decrease
RVI	regulatory volume increase

PEMBANGUNAN DAN PENCIRIAN TEH HERBA NUTRASEUTIKAL DARIPADA DEDAUN *POLYALTHIA LONGIFOLIA*

ABSTRAK

Pembangunan teh herba nutraseutikal daripada tumbuhan ubatan asli telah mendapat populariti kerana banyak manfaat kesihatannya. *Polyalthia longifolia* didokumenkan dengan baik untuk sifat etnoperubatannya yang berharga, tetapi tiada produk komersial yang sedia ada berdasarkan tumbuhan ini. Oleh itu, teh nutraseutikal MyAyush® yang baharu telah dibangunkan dalam kajian ini daripada daun *P. longifolia*. Pada mulanya, untuk menetapkan piawaian bagi daun *P. longifolia* dan memastikan kualiti teh MyAyush®, penilaian analisis proksimat, fitokimia, FTIR dan logam berat telah dijalankan. Selepas itu, penilaian deria, antioksidan, antikanser, dan aktiviti antitumorigenik teh MyAyush® juga dinilai secara *in vitro*. Analisis proksimat, fitokimia dan logam berat mendedahkan kehadiran komposisi proksimat yang penting (6.9% abu, 25% serat kasar, 4.5% lemak mentah, 18.9% protein kasar, 6.6% kelembapan, dan 63.1% karbohidrat), 32 fitokimiaserta kehadiran merkuri, kadmium, plumbum dan arsenik dalam kuantiti yang rendah dan dalam had yang dibenarkan telah ditemui. Penyeragaman teh MyAyush® dilakukan dengan mengukur penanda kimia rutin, yang ditemui dalam kepekatan 1.2 μg/mL (0.12%). Selain itu, analisis FTIR menunjukkan kehadiran sepuluh puncak graf dalam julat 3500 hingga 400 cm^{-1} untuk pelbagai kumpulan berfungsi tertentu. Keputusan penilaian deria menunjukkan bahawa purata tahap penerimaan umum teh MyAyush® (6.6) adalah hampir sama dengan tahap penerimaan (7) teh komersial yang sedia ada. Ujian antioksidan in vitro bagi ekstrak air teh MyAyush® mempamerkan aktiviti bergantung kepada dos dan setanding dengan antioksidan komersial standard. Menariknya, kandungan fenolik dan

flavonoid dalam ekstrak berair teh MyAyush® mempunyai korelasi yang ketara (P < 0.05) dengan kapasiti antioksidan. Tambahan pula, ekstrak berair teh MyAyush® boleh menghalang sel HeLa dengan nilai IC_{50} 1.24 mg/mL tanpa menunjukkan kesan sitotoksik terhadap sel Hs27 bukan kanser. Penyiasatan morfologi sel HeLa yang dirawat dengan kepekatan IC_{50} ekstrak air teh MyAyush® mendedahkan ciri morfologi apoptosis yang menonjol. Ujian pemeluwapan kromatin nuklear telah dijalankan dengan kepekatan ½×IC_{50}, IC_{50} dan 2×IC_{50} ekstrak berair teh MyAyush® untuk menentukan indeks apoptosis. Ia mendedahkan indeks apoptosis dalam cara yang bergantung kepada dos berdasarkan perubahan selular dan nuklear yang diperhatikan semasa rawatan. Kajian *in vitro* selanjutnya menunjukkan bahawa ekstrak berair teh MyAyush® menghalang penghijrahan sel HeLa dengan ketara (P < 0.05) apabila dirawat dengan kepekatan IC_{50} ekstrak berair teh MyAyush®. Tambahan pula, dalam ujian pembentukan koloni, kepekatan ½×IC_{50}, IC_{50} dan 2×IC_{50} yang diuji bagi ekstrak berair teh MyAyush® dengan ketara (P < 0.05) menghalang pembentukan koloni sel HeLa berbanding kawalan yang tidak dirawat. Analisis sitometri aliran bagi ujian Annexin V/Propidium Iodide memastikan induksi apoptosis dalam sel HeLa yang dirawat ekstrak air teh MyAyush® dalam 24 jam. Kesimpulannya, penemuan kajian ini menunjukkan bahawa teh MyAyush® daripada daun *P. longifolia* mempunyai nilai terapeutik yang tinggi dan boleh berfungsi sebagai agen kemopencegah hijau berasaskan produk semula jadi yang berkesan.

DEVELOPMENT AND CHARACTERISATION OF NUTRACEUTICAL HERBAL TEA FROM *POLYALTHIA LONGIFOLIA* LEAVES

ABSTRACT

The development of nutraceutical herbal tea from native medicinal plants has gained popularity due to its numerous health benefits. *Polyalthia longifolia* is well documented for its valuable ethnomedicinal properties, but no commercial products are available based on this plant. Therefore, the new MyAyush® nutraceutical tea was developed in this study from *P. longifolia* leaves. Initially, to establish the standards for the *P. longifolia* leaves, which can be used to correctly identify the plant in the future and ensure the quality of MyAyush® tea, the evaluation of proximate, phytochemical, FTIR and heavy metals analyses were conducted. Subsequently, the sensory evaluation, *in vitro* antioxidant, anticancer, and antitumorigenic activities of the MyAyush® tea were also assessed. The proximate, phytochemical, and heavy metals analyses revealed the presence of crucial proximate composition (6.9% ash, 25% crude fibre, 4.5% crude fat, 18.9% crude protein, 6.6% moisture, and 63.1% carbohydrates), 32 phytochemicals, as well as the presence of, mercury, cadmium, lead and arsenic in low quantities and within the permissible limit. The standardization of MyAyush® tea was performed by quantifying the rutin chemical marker, which was found in a concentration of 1.2 µg/mL (0.12%). Besides, the FTIR analysis showed the presence of ten peaks' areas in the range of 3500 to 400 cm^{-1} for various specific functional groups. The sensory evaluation results indicated that the average general acceptance level of MyAyush® tea (6.6) was close to the acceptance level (7) of the commercially available tea. The *in vitro* antioxidant assays of MyAyush® tea aqueous extracts exhibit a dose-dependent activity and are comparable to the standard

commercial antioxidant. Interestingly, the phenolic and flavonoid contents of MyAyush® tea aqueous extracts are significantly ($P < 0.05$) correlated with antioxidant capacity. Furthermore, MyAyush® tea aqueous extract can inhibit the HeLa cells with an IC_{50} value of 1.24 mg/mL without exhibiting cytotoxic effects against the noncancerous Hs27 cells. The morphological investigation of HeLa cells treated with the IC_{50} concentration of MyAyush® tea aqueous extract revealed prominent morphological characteristics of apoptosis. The nuclear chromatin condensation assay was conducted with the ½×IC_{50}, IC_{50} and 2×IC_{50} concentrations of MyAyush® tea aqueous extract to determine the apoptotic index. It revealed an apoptotic index in a dose-dependent manner based on the observed cellular and nuclear alterations upon treatment. Further *in vitro* studies showed that MyAyush® tea aqueous extract inhibited HeLa cell migration significantly ($P < 0.05$) when treated with the IC_{50} concentration of MyAyush® tea aqueous extract. Furthermore, in the colony formation assay, the tested ½×IC_{50}, IC_{50} and 2×IC_{50} concentrations of MyAyush® tea aqueous extract significantly ($P < 0.05$) inhibited colony formation of HeLa cells compared with the untreated control. Flow cytometric analysis of Annexin V/Propidium Iodide assay confirmed the induction of apoptosis in MyAyush® tea aqueous extract-treated HeLa cells in 24 hours. In conclusion, the current study's findings showed that developed MyAyush® tea from the *P. longifolia* leaves possess high therapeutic values and could serve as an effective natural products-based green chemopreventive agent.

CHAPTER 1

INTRODUCTION

1.1 Overview and Rationale of the Study

The onset of the industrial age has led to significant changes in human lifestyle, including the adoption of fast-paced eating habits with less nutritious but more convenient meals, longer work hours, and increased psychological stress. The increased use of chemicals, heavy metals, electromagnetic waves, and other man-made materials has resulted in soil and food contamination, as well as air and water pollution. As a consequence of these changes, there has been a rise in the prevalence of various health issues, such as diabetes, obesity, vascular diseases, physiological problems, and other degenerative conditions. In addition, there has been a significant rise in medical treatment expenses due to the growing demand for healthcare. The 21st century has brought a focus on the quality of life and an increase in life expectancy, leading to heightened awareness about health. People are increasingly mindful of their diet as they believe it can directly impact their well-being. To enhance their quality of life, many individuals have turned to a diet that incorporates more fruits, vegetables, and plant-based foods, as well as using dietary supplements or nutraceuticals, and exploring nutritional therapy or phytotherapy as alternatives to chemotherapy or radiotherapy (Berger *et al*., 1996; Mollet and Rowland, 2002; Bagchi *et al*., 2004; Siró *et al*., 2008; Vicentini *et al*., 2016). Consequently, as the market for nutraceuticals, phytonutrients, and their medicinal applications has expanded, so have its producers, marketers, and affiliated licensed professionals.

Since ancient times, natural items, primarily plants and their parts have been utilized to treat illnesses. In the past, herbal medicines were crucial to maintaining human life and health. Almost all of the leaves, flowers, fruits, barks, roots, and

rhizomes that are frequently consumed as food and drink, are rich in beneficial phytochemicals (Tipduangta *et al*., 2019). Chinese people were aware that tea may treat, protect and prevent several human diseases as early as 400-5000 years ago (Mair *et al*., 2009). Tea is now consumed by hundreds of millions of people worldwide. Tea is an aromatic beverage that was first discovered in 2700 B.C. and has been used for centuries (Lu, 2021). Tea has attracted much consumer interest over time, which has resulted in the processing of several tea varieties. Green (non-fermented), black (fermented) and oolong (semi-fermented) teas are typically regarded as the three major tea types, each of which is characterized by the way the harvested leaves are processed. There is a lot of evidence from research reports that tea consumption may reduce the risk of cardiovascular and human debilitating diseases (Khan and Mukhtar, 2013; Reygaert, 2017). For consumers who drink tea, the tea-based products market was encouraged by this scientific evidence to improve tea processing with an emphasis on food safety and to maintain excellent health standards. Numerous tea-based products with a high caffeine concentration reached the market to satisfy this rising demand (psychoactive drug naturally present in tea) (Temple *et al*., 2017). If we exclude water, people's cheapest beverage is tea. Since ancient times, drinking tea has been seen as a practice that promotes health. There is scientific evidence for this view provided by various current medicinal research findings. With each new research that is published in the academic research literature, the data proving the health benefits of drinking tea gets stronger (Khan and Mukhtar, 2013). However, the scientific community has noted that drinking tea, which contains a lot of caffeine, has a negative impact on human health. Therefore, it is essential to develop natural, caffeine-free beverages to overcome this issue. Herbal teas are a recent fad produced by brewing plant leaves, seeds, or roots with spices (Sridhar and Linton Charles, 2019).

Among medicinal plants, green tea offers a variety of health advantages. Since ancient times, people have used fresh and dried medicinal plants to make tea, which is widely consumed for its tasty flavour and most importantly, for its alleged health advantages. However, as customers' awareness of their health has increased, plant-based health products, such as herbal teas, have emerged. Additionally, the advantages of herbal teas for health and the most recent developments in the herbal tea industry are reviewed exclusively. In the United States (US), the European Union (EU), Japan, China, Brazil and other nations, numerous plant-based herbal teas have been developed and approved. The beverage industry has only lately begun creating herbal teas, and because of their alleged therapeutic, anti-aging, and functional qualities, more herbal tea products are anticipated to be released for health-conscious consumers (Sridhar and Linton Charles, 2019).

Therefore, the development of plant-mediated nutraceutical tea from native medicinal plants has become the predominant approach in tea production due to its variety of health benefits. *Polyalthia longifolia* var. Angustifolia Thw. (Annonaceae), a highly valued medicinal plant with numerous therapeutic benefits is commonly found throughout Malaysia and has traditionally been used as a tonic and fever-reducing agent (The Wealth of India, 1969). Locally in Malaysia, Glodogan tiang is the name given to *P. longifolia*. *P. longifolia* is a mast tree native to Sri Lanka that is currently grown in tropical areas of India along roadsides and gardens for its attractive appearance. It has linear lanceolate leaves that are 1 to 1.5 cm broad (Bose *et al*., 1998). The studies conducted by Jothy et al. on *P. longifolia* methanolic extract showcased its antioxidant properties and demonstrated its diverse protective capabilities. These encompassed hepatoprotection and radioprotection, along with its genoprotective effects (Jothy *et al*., 2012; Jothy *et al*., 2013; Jothy *et al*., 2016). Subsequently, Jothy

et al. (2013) specifically highlighted the innocuousness of *P. longifolia* to animals, reinforcing its potential as a safe therapeutic agent. Additionally, Vijayarathna (2016) corroborated its potential as an anticancer agent, while Cilwyn (2022) further emphasized its utility as an antitumor and anticancer agent. Concurrently, Hemagirri and Sasidharan (2022a; 2022b) identified its promising antiaging attributes.

These investigations, conducted through both *in vitro* and *in vivo* studies, elucidate the comprehensive scope of *P. longifolia*'s benefits. Notably, Jothy et al.'s comprehensive study included a critical acute oral toxicity assessment. This study administered a single oral dose of up to 5000 mg/kg body weight of *P. longifolia* leaf extract to female albino Wistar rats, demonstrating the extract's safety, even at relatively higher doses (Jothy *et al*., 2016). This further corroborated its benign nature and affirmed its potential for safe use in therapeutic applications.

These compelling findings provide sufficient justifications for the development of a *P. longifolia* leaves-based herbal tea. The diverse range of protective effects observed in the studies, coupled with its safety profile even at higher doses, highlights its potential as a valuable component in herbal tea formulations aimed at promoting health and well-being.

Additionally, *P. longifolia* also offers a low-cost but high-quality source of polyphenols and antioxidants as natural resources (Jothy *et al*., 2012; Jothy *et al*., 2013). All the previously mentioned therapeutic characteristics of *P. longifolia* are crucial characteristics of good plant-based green tea. Numerous recent research publications also suggested that green tea with high-quality polyphenols may lower cancer risk. Additionally, numerous studies have revealed a negative correlation between tea drinking and the risk of developing specific cancers (Khan and Mukhtar, 2013). Notably, the latest findings showed that *P. longifolia* leaves exhibited

anticancer activity while concurrently displaying radioprotective activity against healthy cells (Jothy *et al*., 2016; Vijayarathna, 2016). Therefore, this will be the unique feature of future *P. longifolia* leaves-based tea which may have the potential to reduce the risk of various types of cancers in humans while concurrently protecting the intact cells. Despite the apparent health benefits of *P. longifolia*, there are relatively no commercial products of this plant available in Malaysia, and the plant remains underutilized, although widely reported its remarkable medicinal properties and traditional usage. There is an urgent need to develop *P. longifolia* leaves into usable products such as tea to reach society as a potential nutraceutical. Therefore, this study aimed to create an herbal tea from the leaves of *P. longifolia* local medicinal plant as a natural ingredient. Hence, it is essential to conduct an in-depth study to determine the leaves proximate analysis and to determine the chemical composition and sensory evaluation of *P. longifolia* leaves-based tea infusion. Moreover, phytochemical analysis and evaluation of some biological activities of *P. longifolia* herbal tea infusion are important since no previous studies have evaluated the antioxidants, cytotoxic activities and anticancer potential of the *P. longifolia* leaves aqueous extract.

1.2 Objectives

The current study was designed with the following objectives:

1. To develop a tea product using *Polyalthia longifolia* leaves.
2. To generate proximate analysis data for *Polyalthia longifolia* tea leaves.
3. To determine the chemical composition of *Polyalthia longifolia* tea leaves aqueous extract.
4. To conduct a sensory evaluation of *Polyalthia longifolia* tea leaves infusion.
5. To evaluate the antioxidant activity of *Polyalthia longifolia* tea leaves aqueous extract.
6. To evaluate the *in vitro* cytotoxicity and anti-cervical cancer potential of *Polyalthia longifolia* tea leaves aqueous extract

1.3 Workflow

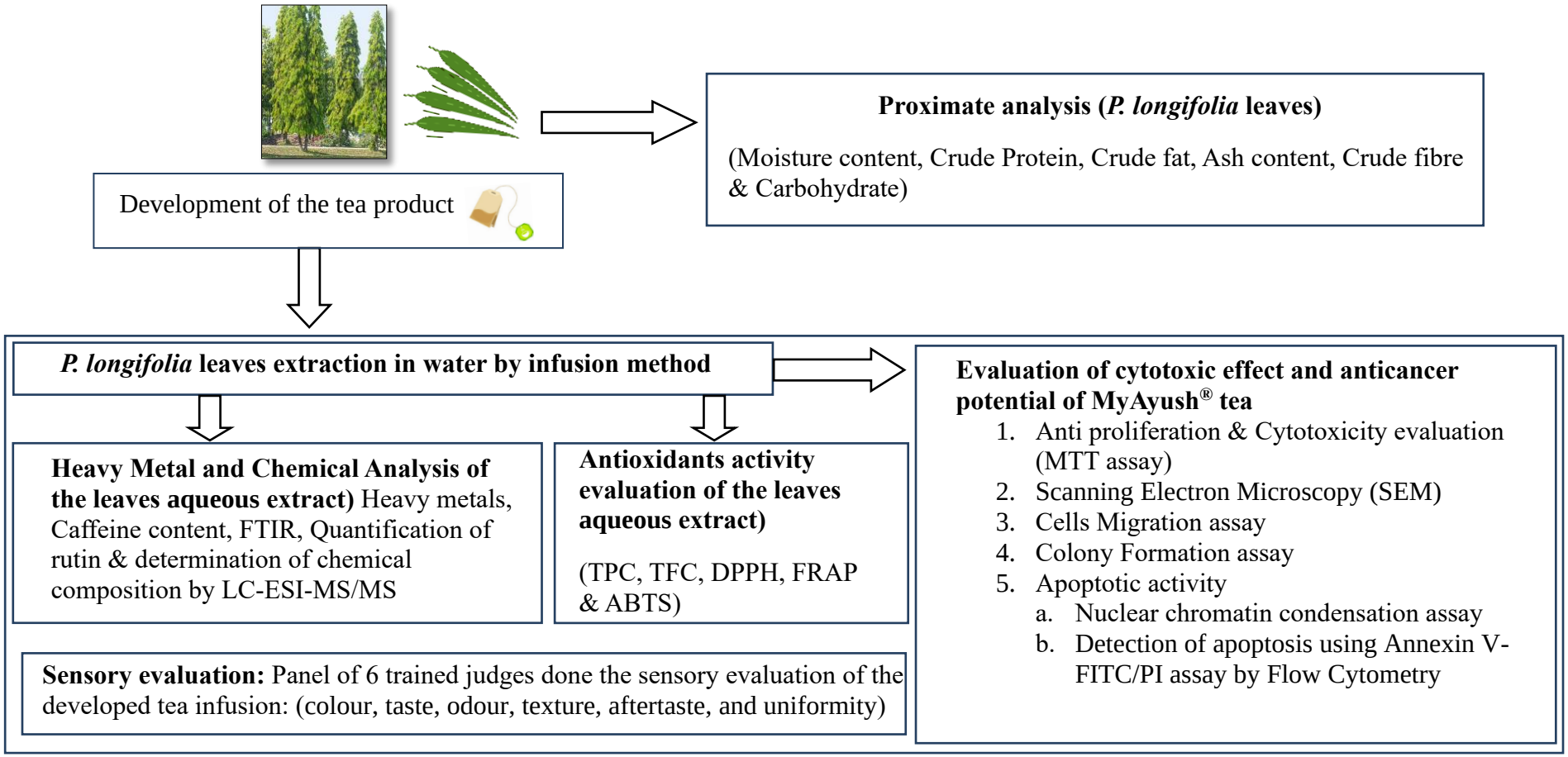

Figure 1.1: Workflow of the overall research

CHAPTER 2

LITERATURE REVIEW

2.1 Nutraceuticals

Plants are a valuable source of medications and human nutrition. The concepts of health, agriculture and food have experienced a significant transformation due to the rapid advancements in the fields of medicine, nutrition, and plant biotechnology. Recent developments in the fields of nutrition and medical sciences have generated considerable attention from the public and healthcare experts towards natural products and meals that enhance well-being. This trend has led to the emergence of novel concepts such as nutraceuticals, nutritional therapy, phytonutrients, and phytotherapy (Bland, 1996; Bagchi, 2006). These functional or medicinal foods, phytonutrients, and phytomedicines have the potential to lower side effects and healthcare costs while simultaneously having a favourable impact on immune function, general health, and the prevention of certain diseases (Ramaa *et al*., 2006). These concepts trace their origins back three thousand years to an ancient idea. Hippocrates, the renowned founder of modern medicine, articulated the notion that "Let food be thy medicine and medicine be thy food," foreseeing the inherent link between nourishing meals and their medicinal benefits (Bagchi, 2006). The accuracy of this adage is still widely acknowledged and recognized today. The definition of these items, also known as functional foods, dietary supplements, and nutraceuticals, is "any substance that may be considered a food or part of a food that delivers medical and health benefits, including the treatment and prevention of disease." Nutraceuticals may be the best name to describe these partially-food and partially-pharmaceutical items. Stephen DeFelice, the founder and chairman of the Foundation for Innovation in Medicine, based in Cranford, New Jersey, first used this neologism in 1989 (Biesalski, 2001). By

combining the words "nutrient" (referring to nourishing food or food components) and "pharmaceutical" (referring to medical drugs), the term "nutraceutical" is formed. Nutraceuticals are products that may include "natural" ingredients and are intended to treat or prevent diseases, despite not necessarily being universally recognized as safe for consumption (Ross, 2000).

Although research had been conducted on the functional properties of food and its ability to prevent and/or alleviate certain ailments, the term "nutraceutical" was not coined until the 1990s. Nutraceuticals, as defined by DeFelice, include dietary supplements, foods and medical foods that possess specific health benefits in the prevention and/or treatment of various diseases (DeFelice, 1989).

Vitamins, minerals and amino acids are examples of Nutraceuticals, extracts, or food derivatives that may offer health advantages in addition to their nutritional worth (Santini *et al*., 2017); They are commonly known as dietary supplements and provide a combination of both essential nutrients (such as carbohydrates, proteins and fats) and non-nutrient compounds (such as probiotics, prebiotics, enzymatic regulators and phytochemicals) that contribute to overall nutrition (Bergamin *et al*., 2019). Recent scientific research has shown that food can be a powerful tool in safeguarding the body and maintaining a healthy state (Santini *et al*., 2017). Nutrition has been found to have both positive and negative correlations with a variety of degenerative, autoimmune, and neoplastic disorders. The relationship between nutrients and organisms can have a significant impact on an individual's overall health and susceptibility to disease throughout their lifetime (DeFelice, 1989). Furthermore, research has indicated that nutraceuticals may be beneficial in conjunction with medications for managing chronic illnesses or minimizing unwanted side effects. The idea that food can be utilized as a form of medicine and that certain foods can have

therapeutic benefits for human health was first conceptualized by Hippocrates in the 19th century with his philosophy of "let food be thy medicine and medicine be thy food".

Phytochemicals that are bioactive, such as terpenoids, alkaloids and various polyphenols (including flavones, anthocyanins, isoflavones, flavanols, ellagic acid, stilbenes, and more) are important sources of nutraceutical ingredients (Jain and Ramawat, 2013). Phytochemicals are natural substances present in plants that possess advantageous pharmacological properties and can positively impact human health. These effects include acting as anti-inflammatory, antifungal, antioxidant, antibacterial, chemopreventive, anti-allergens, neuroprotective, hepato-protective, hypotensive and anti-aging agents. These phytochemicals have been found to have therapeutic effects on a range of health conditions such as DNA damage, osteoporosis, diabetes, heart disease and cancer (Jain and Ramawat, 2013; Caponio *et al*., 2022).

The increased awareness of health problems and concerns among the public has led to a growing market for nutraceuticals, which are bioactive substances derived from food that have properties that promote health and prevent diseases. Nutraceuticals are becoming a significant segment of both the pharmaceutical and food industries due to their beneficial effects (Dikmen and Filazi, 2016). Over the past decade, the scientific community's interest in nutraceuticals has increased significantly, as evidenced by the publication of over 8,000 articles. The increased attention towards nutraceuticals is a result of the worldwide recognition of their potential positive impacts on health. Recent research has demonstrated that nutraceuticals can prevent a range of diseases including Alzheimer's, allergies, eye and cardiovascular diseases, Parkinson's, cancer, diabetes and obesity. Additionally, they can assist in managing inflammation and modulating the activity of the immune system (Nasri *et al*., 2014).

Therefore, there has been a lot of interest in nutraceuticals, which presents new prospects for the creation of creative products that will satisfy customer demands for meals that promote health (Chopra *et al*., 2022). In 2021, the worldwide market for nutraceuticals was valued at $289.8 billion, and it is expected to rise to $438.9 billion by 2026. The annual growth rate of nutraceutical compounds for the year 2021 is projected to be 8.7%, primarily due to the expanding availability of commercially accessible nutraceuticals and their diverse range of applications (Future Market Insights, 2022; Tsiaka *et al*., 2022). Additionally, the nutraceutical market is anticipated to grow in the aftermath of the COVID-19 pandemic due to the potential advantages these products may have for the health of the human immune system (Tsiaka *et al*., 2022). Additionally, there are now more patents based on nutraceuticals, showing the critical importance of these products around the world (Daliu *et al*., 2018).

2.1.1 Nutraceuticals Health Benefits

Epidemiological studies conducted on large populations consistently demonstrate that diet plays a significant role in both the development and progression of chronic diseases such as type 2 diabetes, cataracts, gallstones, cardiovascular diseases, neurological disorders and certain types of cancer such as gastrointestinal cancer. It is well-established that dietary choices and food intake have a direct impact on health and the risk of developing certain diseases (Calvani *et al*., 2020). Humans have developed a comprehensive and cooperative system of antioxidant defence mechanisms against hazardous oxygen intermediates through eating, according to biochemical and epidemiological evidence. Increased consumption of antioxidants through food may reduce the incidence of illnesses like cancer (Benzie, 2000). Micronutrient supplements, functional foods, and nutraceuticals can inhibit cancer cell

growth by stopping their proliferation and inducing apoptosis (Tripathi *et al*., 2005). Many types of organic foods have shown promise in the prevention and treatment of cancer, and they are believed to be a potent strategy (Zheng *et al*., 2016). Nutraceuticals derived from natural sources and included in the diet, like carotenoids, have the potential to exhibit anticancer properties by increasing gap-junctional communication *in vitro* via upregulation of connexin 43 (Cx43), (Hiramatsu and Yoshikawa, 2005) or flavonoids, which can regulate the phases I and II of xenobiotic detoxification, and vitamin E, which can inhibit protein kinase C, an enzyme involved in the development of several cancers (Aggarwal *et al*., 2010).

2.1.2 Categories of Nutraceuticals

Nutraceuticals are dietary supplements employed as biological treatments to manage symptoms, prevent diseases and enhance overall well-being. Nutraceuticals can be divided into three broad categories as follows (Hathcock, 2001):

1. Nutrients: compounds that have been proven to have nutritional benefits, such as vitamins, minerals, fatty acids and amino acids.
2. Dietary supplements: chemicals produced from different sources (such as pyruvate, chondroitin sulphate and steroid hormone precursors) that are used for specialized purposes, like meal replacements, weight-loss supplements, and sports nutrition.
3. Herbals: extracts and herbs or other botanical items. Herbs are as old as human civilization and offer a vast array of treatments for both acute and chronic illnesses. The earliest written tradition for using natural treatments, known as "Ayurveda" in India, includes several efficient ways to guarantee health care.

Essential components of therapeutic herbs include numerous nutraceuticals (Prabu *et al*., 2012).

2.2 Tea

2.2.1 Tea History and Origin

By definition, tea is a beverage made by infusing young leaves, leaf buds and internodes of *Camellia sinensis* or *Camellia assamica* kinds of tea plant (Hicks, 2001). According to ancient legend, it is believed that Shen Nung, the second emperor of China, accidentally discovered tea around 2737 BCE (Pan *et al*., 2022). One day, he was unwinding in the yard with a cup of boiling water after a substantial supper. A few leaves from a neighbouring tree fell into the cup at that same moment. He took a sip without realizing it. The habit of drinking tea was established as a result of his enjoyment of its flavour and perception that it significantly reduced the amount of discomfort he was experiencing at the time. According to an Indian myth, Buddha began to feel sleepy in the fifth year of a seven-year sleepless meditation. He quickly plucked a few leaves from a nearby shrub and began chewing them. Miraculously, his fatigue dissipated. The plant happened to be an untamed tea tree (Selvakumar and Jeyaselvam, 2012).

Father Jasper de Cruz, a Portuguese Jesuit missionary, was the first European to experience tea and write about it in 1560. The Dutch brought several teas and tea customs to New Amsterdam city around 1650 (which eventually transformed into the city of New York). In 1657, at Garway's Coffee House in London, England, the first tea was introduced and consumed as a beverage with health-promoting properties. The first retail tea was offered by John Horniman in lead-lined, sealed containers in 1826. Twinings of England, a company that originated in England and was founded in 1706, has a rich history of tea blending and trading. It is recognized as one of the world's

oldest and most esteemed tea companies. In 1870, Twinings of England began blending tea to achieve consistency in their tea products. Richard Blechynden, an Englishman, invented iced tea in 1904 amidst a period of scorching temperatures at the St. Louis World Fair. Tea bags were accidentally invented by tea importer Thomas Sullivan of New York in 1908 when he supplied tea to customers in small silk bags that were inadvertently steeped whole. In 1953, the first instant tea in the world was unveiled. Today, green tea is drunk in Asia, while black tea is consumed in North Africa, North America and Europe (Serafini *et al*., 2012).

2.2.2 Tea Trade History

After being consumed in China for thousands of years as a medicinal beverage made by steeping fresh leaves in boiled water, tea became a common beverage and the cultivation and processing of tea started about the third century C.E. In 350 C.E., the earliest description of how to plant, prepare and drink was published. Tea cultivation has a long history, with the first tea seeds being introduced to Japan around 800 AD, and agriculture based on tea establishing itself by the 13th century. Tea cultivation was introduced to the Formosa (Taiwan) island in 1810 by Chinese immigrants from Amoy. The Dutch brought Japanese seeds and workers to Java in 1826 and introduced Chinese seeds, labourers, and equipment to the highlands near the border between the Indian state of Assam and Burma in 1833. Tea trees were discovered in this region in 1824. In 1836 and 1867, respectively, the cultivation of tea in India and Ceylon (Sri Lanka) was introduced by the British, initially using Chinese seeds but eventually switching to Assam plant seeds (Sivasubramaniam, 2022).

In 1610, the Dutch East India Company successfully delivered the initial consignment of Chinese tea to Europe, while in 1669, the English East India Company

transported Chinese tea to the London market. Over time, tea produced on British estates in Ceylon and India also became popular. By the late 19th and early 20th centuries, tea was being grown in non-Asian nations such as Russian Georgia, Sumatra, and Iran, as well as several African countries including Malawi, Natal, Congo, Uganda, Kenya, Mozambique and Tanzania. South American countries like Argentina, Brazil, and Peru, as well as Queensland in Australia, also began growing tea (Sivasubramaniam, 2022).

A wide variety of teas are produced throughout the Asian region. As a result, Asia now holds a significant share in every global tea importing market, owing to its reputation for producing high-quality teas that are well-regarded in global markets. Tea is also extensively produced in the Near East, South America, and Africa, contributing to significant tea production worldwide. It is consumed regularly throughout the day by large populations in various regions including Asia, the UK, the EU, the Middle East, Africa, and the Commonwealth of Independent States (CIS) (Hicks, 2001).

2.2.3 Common Types of Tea

The most commonly used tea plant is *Camellia sinensis*, which is an evergreen shrub that has several variations, with the Indian Assam tea being the most well-known (*C. sinensis* var. assamica Kitamura). To create the traditional beverage, dried young leaves and leaf buds are steeped in boiling water. Although the tea plant is native to North India, Southern China, Cambodia and Myanmar, China is considered to be the country that introduced tea to the rest of the world (Hicks, 2001).

There are three main types of Camellia tea, which are commonly classified as Oolong, Green and Black, even though many countries produce blends of these types.

The key factor that distinguishes them is the process of fermentation, which involves enzymatic and oxidative changes in the tea leaves. Oolong tea is partially fermented, green tea is unfermented and black tea is completely fermented. Black tea is the most widely traded type and produces a rich, amber-coloured beverage with a full flavour that is not bitter. Two examples of black teas are Pekoe and Orange Pekoe, which are prepared from the very top leaves of the tea plant. The term "Pek-ho" in Chinese refers to the silver-tipped Assam teas. Orange Pekoe is typically sourced from Sri Lanka or India, while Pekoe tea is made from even smaller leaves and is imported from Sri Lanka, India or Indonesia (Hicks, 2001).

However, reports from India suggest that the leaves of five other mangrove species, including *Ceriops decandra* (Griff), *Bruguiera cylindrical* (L) Bl, Ding Hou, *Rhizopora apiculate* Blame, *R. lamarckii* Montr and *R. mucuonata* Lam, and Blame can be used as alternative sources of tea (Kathiresan, 1995). The leaves of various plants, including *Sorbus aucuparia*, *Epilobium anguistifolium*, *Fragaria vesca*, *Filipendula ulmaria*, and *Rubus idaeus*, have been formulated to make tea by previous workers in Europe (Julkunen-Titto *et al*., 1988). They contain several aromatic components that have medicinal properties for humans. Herbal tea might be a better description of these infusions of different plants. A variety of herbal teas are available in many Asian countries, which are made by brewing plant leaves or other parts such as flowers. One such plant is *Gymnema sylvestre* from the Asclepiadaceae family, which is commonly found in India and is used to produce a nutritious and healthy herbal tea with various medicinal properties. In general, the popularity of herbal teas is increasing nowadays (Hicks, 2001).

2.2.4 Herbal Tea

Herbal tea, or "tisanes," refers to the infusion of plants other than *Camellia sinensis*. Although herbal tea looks like tea and is prepared similarly to tea, it is not tea at all. Tisanes or herbal teas are different from traditional teas, which are made from the Camellia Sinensis plant. These herbal teas are created by infusing seeds, dried leaves, nuts, grasses, barks, flowers, fruits, or other botanical ingredients to produce their distinctive flavour and health benefits. Unlike other types of tea, herbal teas do not contain caffeine and are easy to drink because of their pleasant taste. These teas can be made using a single herb or a blend of herbs that are projected to provide a specific effect such as rejuvenation, relaxation, or relief from certain conditions (Killedar *et al*., 2017).

Tea made from herbs has been popular for a long time. Ancient Egyptian-era documents have been found that speak of the benefits and usage of herbal tea (Kara, 2009). Before becoming popular as a daily beverage, a herbal beverage is typically appreciated as a medicinal beverage. For example, tea evolved from being a "medication" to being a "drink." (Mellgren, 2001). Not only herbal tea, but authentic tea also undergoes this influence. Around 5000-6000 years ago, in the Shen Nong dynasty of ancient China, tea leaves and other herbs were utilized for medical purposes (Yen and Chen, 1994). Tea drinking first appeared as a beverage in the late Zhou Dynasty (1124-222 B.C.), and it steadily gained popularity in the Qin Dynasty (221-206 B. C.) (Chen 2002).

2.2.5 Health Benefits of Herbal Teas

Along with beverages, fruit and vegetable juices, sports drinks, and teas are considered to be functional foods (Byun and Han, 2004). By definition, functional food lowers the

chance of developing a disease or has a relevant impact on well-being and health. The functional component of a functional food may consist of an essential macronutrient, essential micronutrient, non-essential nutrient, or non-nutritive component (Roberfroid, 1999). Teas, despite having little intrinsic nutritional value, are rich in phenolic chemicals, which are associated with numerous established health benefits (Hamilton-Miller, 1995; Marongiu *et al*., 2004). Haslam (1998) addressed the biological effects of polyphenols, including their anticancer, anti-allergic, antibacterial, antiviral, anti-inflammatory, estrogenic, and immune-stimulating properties. They also have a reputation for having high water solubility.

Due to its growing popularity in the domestic and global markets for health advantages, herbal tea has seen an increase in consumption over the past few years. Numerous research studies have highlighted the numerous health advantages of herbal teas as well as the possibility that drinking them could lessen blood-related irregularities (Sridhar and Linton, 2019; Reis *et al*., 2016). For example, red raspberry leaves tea has been traditionally employed for treating various ailments and, in specific pregnancies, to induce labour when necessary. As indicated in Table 2.1, several researchers have explored the therapeutic advantages of herbal teas. For example, daily consumption of 1 cup (or 0.25 tsp/day for children) of chamomile herbal tea has been found to alleviate stomach discomfort and reduce anxiety. Similarly, drinking one cup of cinnamon herbal tea per day has shown the potential in reducing allergic reactions, LDL cholesterol, and blood sugar levels. For pregnant and breastfeeding individuals, it is recommended to consume 2-3 cups per day of herbal teas such as citrus peel, ginger, orange peel, lemon balm, and rosehip to maintain good health (Sridhar and Linton, 2019). According to research conducted by da Silva *et al*. (2017), the Brazilian berry can be utilized as a tea beverage to harness the benefits of its antioxidant content.

Their findings suggest that consuming herbal teas in recommended dosages may have an impact on various diseases.

Table 2.1: Different types of herbal teas and their consumption doses†

Herbal tea	Consumption doses
Valerian	Infusion: 0.5 to 1 tsp dried root in 1 cup of water. Sleep aid: Drink 1 cup before bed. Anxiety: Drink 1 cup (three times a day: TID).
Peppermint	Infusion: 1 to 2 tsp dried leaves in 1 cup of water for 5 minutes. For Irritable bowel syndrome, consider tablets (200 mg TID).
Nettle	Infusion: 2.5 tsp dried root in 1 cup of water for 5-10 min. Drink 1 cup of tea BID, TID.
Rosemary	Infusion: 2 to 3 tsp crushed leaves in 1 cup of water for 5-10 min. Drink 1 cup of tea TID.
Lemon balm	Infusion: 2 to 4 tsp leaf in 1 cup of water for 5-10 min. Sleep aid: Drink before bed. Anxiety: Drink 1 cup of tea BID and TID.
Ginger	Infusion: 1 tsp root in 1 cup of water, take TID. Migraine: 1 tsp at the start of a headache, repeat in 4 h (max 4 tsp/24 h). Childhood diarrhoea: a piece of ginger root the size of a child's little finger steeped for 5-10 min.
Cinnamon	Infusion: 0.5 to 3 tsp cinnamon bark in 1 cup of water for 5 min. Drink 1 cup of tea daily (may steep black teabag with bark for flavour if desired).
Fennel	Infusion: 1.5 to 4 tsp crushed fruit or seed in 1 cup of water. Take 1 cup of tea TID. Children: 0.04 tsp/lb/day not to exceed the adult dose.
Chamomile	Infusion: 1.5 to 5 tsp dried flower heads in 1 cup of water for 5-10 min. Drink 1 cup of tea TID. Children: 0.25 tsp/lb/day not to exceed the adult dose.
Motherwort	Infusion: 2 to 3 tsp dried stems, leaves, and flowers in 1 cup of water for 5-10 min. Drink 1 cup of tea TID.

†Source: (Sridhar and Linton, 2019)

2.2.6 Herbal Tea Sources Around the World

Herbal teas have long been a staple of the traditional diet, helping to prevent a variety of illnesses. The study cited by Chandrasekara and Shahidi provided documentation of the various medical benefits attributed to the use of herbal teas as a treatment. Generally, each herbal tea was created to provide a particular therapeutic benefit and was made from a variety of herbal sources (Chandrasekara and Shahidi, 2018). For example, Asians produce herbal teas from many sources, while people from other regions, such as Africa and Europe, also prepare herbal teas from various sources, as indicated in Table 2.2.

Table 2.2: Different sources of herbal teas around the world†

Place	Sources
Asia	*Centella asiatica*, Arabian jasmine, Balsam pear, barley grass, guava, hardy rubber tree, Japanese persimmon, Jobs tears, Wolof berry tea, *A. marmelos* (bael) and Tanner's Cassia (*Cassia auriculata*)
Africa	African rooibos (*Aspalathus linearis*), borututu (*Cochlospermum angolensis*) and honeybush tisanes
South America	Yerba mate (*Ilex paraguariensis*) and Kombucha tea
Europe	*Chamomilla recutita*, *Matricaria chamomilla*, *Chamaemelum nobile* and Peppermint tea (Mentha piperita leaves)

†Source: (Chandrasekara, Shahidi, 2018)

2.2.7 Herbal Tea Market

In recent times, around 80% of the global population has been dependent on traditional medicine for their primary healthcare needs, which often includes the use of plant extracts, usually in the form of water-based solutions (Ekor, 2014). The public's interest in herbs that were used in traditional medicine has grown due to their long history of use and widespread acceptance. Making herbal tea from the plant is one of the easiest ways to reap its benefits. Herbal teas have become more popular worldwide because they are believed to have antioxidant activity and aroma, which can have a relaxing effect on the mind (Aoshima *et al*., 2007). It has been noted that the development of the health sector, which is directly related to the ability of herbal teas to burn fat, has significantly aided in the expansion of this market (Future Market Insights, 2022).

The perception of herbal teas is transforming, and a notable trend is the cultivation of herbs in home gardens to prepare fresh herbal drinks. This phenomenon is increasingly prevalent not only among Asian families but also among American and European nations. Extensive media coverage of homemade teas in the aforementioned regions may serve as evidence that herbal teas are now widely consumed, including in emerging nations. This suggests that the popularity and acceptance of herbal teas have expanded significantly, potentially due to increased media exposure. Numerous plant items have been utilized throughout Africa for millennia as herbal teas, as well as common local drinks, enjoyed for both their refreshing properties and their medical and health benefits. Herbal teas have long been a popular way for middle-class families to prevent disease. An investigation into the public's knowledge and perceptions of the effectiveness, safety, and justification for consuming herbal teas in Malaysia has previously been carried out by a research team from Universiti Sains Malaysia.

According to the results, the majority of Malaysians (57.0%) regularly drink herbal tea and think that these beverages are safer to ingest and have fewer adverse effects than the commercially available conventional treatments. The locals also think that drinking herbal teas can help promote health, slim down, increase energy, and prevent and treat ailments, in addition to having beauty benefits (Hassali *et al*., 2009).

The demand for herbal tea increased at a 5.5% value CAGR (Compound annual growth rate) from 2016 to 2021. The global tea industry is expanding quickly, especially for herbal teas, as the trend toward health and well-being continues to increase. The market for herbal tea is anticipated to reach $3,700 million in 2022, and during the forecast period of 2022 to 2032, sales are anticipated to increase at a CAGR of 7.1%, reaching $7,339.9 million. By 2028, the total global market for herbal tea is anticipated to reach $4.14 billion (Future Market Insights, 2022).

The report predicts that the Asia Pacific region will have the largest and fastest-growing herbal tea market over the projection period. The consumption of herbal tea in nations like China and India is anticipated to contribute the most to the regional industry as it grows. Herbal tea holds a promising and established market due to its recognized anti-ageing benefits. Presently, tea bags are commonly produced with biodegradable or compostable outer packaging, effectively reducing the environmental impact of atmospheric carbon dioxide. To further enhance overall sales, a ready-to-drink variety is also being introduced into the market. Innovative marketing strategies and celebrity endorsements are anticipated to play a significant role in expanding the market in the coming years (Future Market Insights, 2022).

Consumer priorities have altered as a result of the Corona Virus Pandemic, according to research conducted by Accenture. They projected those consumers will continue to embrace new behaviours and routines over time, such as a rising emphasis