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Plant Extracts

Edited by Aman Dekebo

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Contributors
Aman Dekebo, Ganesh Kumar Anbazhagan, P. Sankarganesh, Baby Joseph, Helen Sheridan, Jinfan Wang, Astrid Sasse, Ryszard Amarowicz, Wojciech Rybiński, Magdalena Karamać, Katarzyna Sulewska

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Meet the editor

Aman Dekebo, PhD is an associate professor in the Department of Applied Chemistry at Adama Science and Technology University, Ethiopia. He obtained his first and second PhDs in Chemistry from Addis Ababa University, Ethiopia, in 2002 and Bio-organic Chemistry from Ehime University, Japan, in 2007, respectively. From 2017 to 2019, during his sabbatical leave, he worked as a researcher at Andong National University, South Korea. His research interests include isolation and characterization of bio-active compounds, chemical ecology and apiculture. Dr. Dekebo has authored more than 45 peer-reviewed scientific papers, book chapters in press, and one book. He also serves as a reviewer for many reputable journals. He received awards and certificates for his research from the Korean Society of Applied Entomology and the Korean Apiculture Association.

Contents

Preface

The natural products derived from medicinal plants have proven to be an abundant source of bioactive compounds, many of which have been the basis for the development of new, leading bioactive molecules in the drug discovery program. With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to the discovery of novel anti-infective chemicals. Currently, information about medicinal plants is contained in somewhat disjointed reports about the helpful qualities and toxic effects of different plant species throughout the world. The aim of this book is to help bring more unity and understanding to this complicated and often contradictory jumble of information.

Some of the most compelling reasons for writing this book include the following:

- While there has been a great deal of progress made in understanding extracts of plants, a general lack of knowledge and much misinformation remain about natural products in plants and their uses by people.

- Many of the natural products in plants offer us new sources of drugs that have been used effectively for centuries in traditional medicine. Many compounds used in medicine today have original derivatives that were of plant origin.

- Plants are sources of poisons, addictive drugs, and hallucinogens, which have importance in human medicine.

- Many people are interested in using plants parts as traditional medicine, but these people must be made aware of potential harmful effects of such preparations unless properly formulated.

This contributed volume is organized into four chapters. Chapter 1 is an introduction to the uses of plant extracts and some bioactive compounds from medicinal plants and their potential in the drug discovery program. Chapter 2 discusses the practical experience of Chinese traditional medicine and its preparations. China has a long history in effectively using traditional medicine in different ways to treat diseases that cannot be cured by conventional medicine. Chapters 3 and 4 present research findings on bio-activities of different plants extracts. Faba bean extracts, characterized by a high content of phenolic compounds and condensed tannins, are discussed in Chapter 3, while the antiviral activity of the *Phyllanthus* species is evaluated in Chapter 4.

This book is useful to natural product chemists, pharmacologists, pharmacists, research investigators in industry, physicians, nurses, nurse practitioners, and practitioners of integrative medicine; ethnobotanists, organic gardeners, and farmers; and those interested in herbs and herbal medicine. With the growing interest in this field by professionals and the general public alike, we thought it important to produce a book that encompasses as much information as possible on the importance of extracts obtained from medicinal plants in today's world. We hope that this book helps to meet this need.

I'd like to thank all the authors who have made their expert contributions to the realization of this book, which I hope will stimulate interest for further studies in natural product chemistry.

Aman Dekebo
Adama Science and Technology University,
Adama, Ethiopia

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Chapter 1

Introductory Chapter: Plant Extracts

Aman Dekebo

1. Introduction

Humans are dependent on plants for basic needs such as food, clothing, and shelter for centuries [1]. Plants have also been used as arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purposes, stimulants for endurance, and hunger suppression, as well as inebriants and medicines [1]. Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. Relatively 1–10% of plants are used by humans out of estimated 250,000–500,000 species of plants on Earth [2]. Plant-based traditional medicine plays a key role in the development and advancement of modern studies by serving as a starting point for the development of novelties in drug discovery [3]. Various modern drugs were extracted from traditional medicinal plants through the use of plant material following the ethno botanical leads from indigenous cures used by traditional medical systems [4]. In developing countries and rural societies, the use of medicinal plants is both a valuable resource and necessity, and furthermore, it provides a real alternative for primary healthcare systems [5].

Plants were once the primary source of medicines in the world and since then, plants continue to provide humans with new remedies as 50% of all drugs in clinical use in the world are derived from natural products, of which higher plants contribute 25% of the total [5, 6]. The use of plants as medicines predates written human history. Knowledge of plant use was widespread in ancient civilizations. Until the middle of the nineteenth century, plants were the main therapeutic agents used by humans, and even today, their role in medicine is still relevant. It was estimated by the WHO that around 80% of the world population relied on medicinal plants as their primary healthcare source [7] and the demand is increasing in developing countries. For example, traditional medicine (TM) practices have been used for thousands of years by people in Africa, China, India and many other countries [8]. Even in developed countries, plant-based traditional medicines are often termed complementary or alternative medicine (CAM), and their use has increased steadily. For example, in the USA alone, the total estimated that herbal sales for 2005 were $4.4 billion, a significant increase from $2.5 billion in 1995 [9].

The plant chemicals used for arrow or dart poisoning purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. Secondary metabolites can be classified into several groups according to their chemical classes, such as alkaloids, terpenoids, tannins, phenolics, and others [10]. Most of these secondary metabolites are used for plants defense against predators.

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Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society especially for medicinal uses. The medicinal power of these plants is due to their phytochemicals which can cause definite physiological actions on the human body. Phytochemicals are natural compounds which occur in plants such as medicinal plants, vegetables, and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases [11, 12]. Medicinal plants because of their accessibility and affordable health care are an important source of livelihoods for indigenous and rural populations. They are also the source of many active ingredients for modern pharmaceuticals. Between 50,000 and 80,000 flowering plants are used for medicine worldwide, many of them are collected from wild resources [12]. Herb as a group of plants was also used as food (vegetables) and flavors for hundreds of years in many parts of the world. Herbs have been traditionally regarded as natural remedies for common ailments of a human. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines which are antimicrobial sources to cure infections [13]. Conventional antifungal and antibacterial treatment of infections becomes inefficient to cure patients, providing resistance of microorganisms.

Drug	Plant source	Disease to treat	Reference
Galantamine	*Galanthus woronowii* Losinsk	Alzheimer's disease	[14, 15]
Apomorphine	—	Parkinson's disease	[16]
Tiotropium bromide	—	Obstructive pulmonary disease	[17]
Varenicline	—	Smoking cessation	[18–20]
Betulinic acid	Many plants	Anticancer, antibacterial, antimalarial, anti-HIV, anthelminthic, anti-inflammatory, and antioxidant properties	[21, 22]
Combretastatin A4 phosphate	*Combretum caffrum* Kuntze	Anaplastic thyroid cancer	[23]
Huperzine A	*Huperzia serrata* (Thunb. ex Murray) Trevis	Alzheimer's disease	[24]
Ingenol 3-angelate	*Euphorbia peplus* L.	Warts and actinic keratoses	[25]
Morphine	*Papaver somniferum*	Pain	[26]
Protopanaxadiol	*Panax ginseng*	Apoptotic effect on cancer cells	[27]
Harpagoside	*Harpagophytum procumbens* DC	Pain	[28]
Harpagophytum procumbens extract	*Harpagophytum procumbens*	Hip and knee osteoarthritis	[28]
Flavocoxid (Limbrel) blend of flavonoids	*Scutellaria baicalensis* Georgi and *Acacia catechu* Willd	Osteoarthritis	[1]
Ginkgo extract	*Ginkgo biloba* L.	Alzheimer's disease	[29]
Mistletoe extract (Iscador)	*Viscum album* L.	Various cancer	[30]
Sativex extract	*Cannabis sativa* L.	Neuropathic pain	[31]

Table 1.
Compounds and extracts derived from plants used as drugs or on the process of clinical trial stage.

1.1 Plant-derived drugs and various clinical trial stages

Table 1 shows compounds or extracts are drugs or on the process of clinical trial stage derived from plants and their therapeutic use.

2. Uses of plant extracts in food industry

Plants have also been used in the production of stimulant beverages, such as tea, coffee, cocoa, and cola, and intoxicants, such as wine, beer, and kava, in many cultures since ancient times. Tea (*Camellia sinensis* Kuntze) was first consumed in ancient China [1], while coffee (*Coffea arabica* L.) was initially found in Ethiopia from the region called Keffa and then cultivated in Yemen for commercial purposes in the ninth century [32]. The Aztec nobility is used to consume bitter beverages containing raw cocoa beans (*Theobroma cacao* L.), red peppers, and various herbs [32]. The active components of these stimulants are methylated xanthine derivatives, called caffeine, theophylline, and theobromine, major components of coffee, tea, and cocoa, respectively [8].

Studies have shown that a low to moderate consumption of red wine is important for reduction of mortality caused by cardiovascular disease and cancer [33]. This health benefit of wine has been suggested to be due to the presence of a compound called resveratrol present in the skin of grapes [34].

Kava, a beverage made from the root of *Piper methysticum* Roxb., has been a popular intoxicating beverage in Polynesia for centuries [35]. However, in the Western world, kava is not normally consumed directly but has gained popularity as a botanical dietary supplement to ease the symptoms of stress, anxiety, and depression [36]. A study has shown that the anxiolytic activity of kava extract may be mediated in part by the kavalactone and dihydrokavain [37].

3. Extraction methods

Medicinal plants are becoming very important due to their uses mainly as a source of therapeutic compounds that may lead to the development of novel drugs. Most of these compounds such as phenolics and flavonoids have been reported to be important for on health including cancer prevention [38]. High content of phenolic and flavonoids in medicinal plants has been correlated with their antioxidant activities that are important in prevention of the development of age-related disease, especially those related to oxidative stress [39]. Phytochemicals in medicinal plants are very important in pharmaceuticals and cosmeceutical industry [39].

Extraction is the separation of medicinally mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids, and flavonoids using selective solvents through standard procedures [40]. The aim of all solvent extraction methods is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc. The following are the widely used extraction techniques.

3.1 Maceration, infusion, percolation, and decoction

Maceration extraction method is used in wine making and also used for extraction of bioactive compounds from plants [39]. Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for at least 3 days with frequent agitation [40], which is followed by pressing or straining and filtration. In conventional methods, heat is transferred through convection and conduction, and the solvent is selected based on the compound aimed to be extracted [40]. Infusion and decoction use the same principle as maceration but both are soaked in cold or boiled water [39].

Decoction is only suitable for extracting heat-stable compounds, hard plant materials such as roots and barks, and the process usually yielded more oil-soluble compounds compared to maceration and infusion [39].

3.2 Soxhlet extraction or hot continuous extraction

In this method, the sample is ground and placed in a porous bag called thimble made from a strong filter paper or cellulose. The thimble containing the sample is placed in thimble chamber of the Soxhlet apparatus. Extraction solvents are heated in the bottom flask, then vaporized into the sample thimble, condensed in the condenser, and dripped back which result in extraction of plant secondary metabolites in a good yield.

3.3 Microwave-assisted extraction (MAE)

This extraction method uses microwave energy to facilitate partition of analytes from the sample matrix into the solvent [41]. Microwave radiation interacts with dipoles of polar and polarizable materials such as solvent and a plant sample results heating near the surface of the materials and heat is transferred by conduction. Dipole rotation of the molecules induced by microwave electromagnetic disrupts hydrogen bonding enhancing the migration of dissolved ions and promotes solvent penetration into the matrix [42].

3.4 Ultrasound-assisted extraction (UAE) or sonication extraction

This method makes use of ultrasound ranging from 20 to 2000 kHz [40]. The mechanic effect of the ultrasound increases the surface contact between solvents and samples [39]. The ultrasound alters and disrupts the physical and chemical properties of the sample and facilitates release of compounds and enhancing mass transport of the solvents into the plant cells [43].

3.5 Accelerated solvent extraction (ASE)

Accelerated solvent extraction is more efficient solvent extraction method compared to maceration and Soxhlet extraction methods. This method makes use of minimum amount of solvent compared to maceration and Soxhlet extraction methods [39]. In ASE, sample is packed with inert packing material such as sand in the stainless steel to prevent sample from aggregating and blocking of the system tubing [39, 44]. The method controls temperature and pressure for each individual sample and the extraction completes in less than an hour [39].

3.6 Supercritical fluid extraction (SFE)

Supercritical fluid is a substance that shares the physical properties of both gas and liquid at its critical point. Temperature and pressure are the determinant factors that push a substance into its critical region [39]. Supercritical fluid behaves more like a gas but have the solvating characteristic of a liquid. For instance, CO_2 becomes supercritical fluid at above 31.1°C and 7380 kPa. Interest in supercritical-CO_2 extraction is quite interesting due to its excellent solvation power for nonpolar analytes. Additionally, CO_2 is readily available, cheap and has low toxicity [39]. Supercritical-CO_2 has poor solubility for polar compounds. The solubility of supercritical-CO_2 for polar compounds can be enhanced by adding small amount of ethanol and methanol [39].

3.7 Enzyme-assisted extraction (EAE)

In some plants, some phytochemicals in their matrices are dispersed in cell cytoplasm, and secondary metabolites are retained in the polysaccharide-lignin network by hydrogen or hydrophobic bonding and are not accessible with a solvent extraction process [45]. Enzymatic pre-treatment has been considered as an effective way to release bounded compounds and increase overall yield [46]. Specific enzymes such as cellulase, a-amylase, and pectinase added during extraction enhance recovery by breaking the cell wall and hydrolyzing the structural polysaccharides and lipid bodies [46, 47]. There are two approaches for enzyme-assisted extraction method namely enzyme-assisted aqueous extraction (EAAE) and enzyme-assisted cold pressing (EACP) [48]. EAAE methods have been employed mainly for the extraction of oils from various seeds [46, 49–51]. In EACP method, enzymes are used to hydrolyze the seed cell wall of a plant [52].

3.8 Extraction of volatile organic compounds

Distillation is the separation process of the components of a mixture of two or more liquids due to their difference in their vapor pressure. The aims of distillation process are to obtain an essential oil. Hydro-distillation (HD), steam distillation (SD), simultaneous distillation solvent extraction (SDE), microwave-assisted hydro-distillation (MWHD), supercritical fluid (CO_2) extraction (SFE), purge and trap, and solid phase microextraction (SPME) [53] were employed to extract volatile organic compounds from fresh plant parts. Among these techniques, HD, SD, and SDE are classical and conventional methods for sampling bioactive volatile organic compounds (VOCs). Hydrodistillation is the distillation technique carried out either by boiling the plant materials or essential oil with water by creating the steam. The composition of the oil distillates from a mixture of two liquids depends primarily upon the boiling points or the vapor pressure at the distillation temperature.

Problems connected with conventional methods include long extraction times, large amounts of solvents, and multiple steps. Additionally, many unstable volatile organic compounds may be thermally decomposed and degraded during the thermal extraction or distillation. Because of their simplicity, these methods are still in use to extract fragrance-and-aroma oils from plants. Purge and trap, SFE, and SPME have aroused much attention from analysts as they are environment friendly sampling techniques for bioactive VOCs [53].

3.8.1 Purge and trap

Purge and trap also known as dynamic headspace uses ultra-purified inert gas as the carrier gas to pass through samples continuously to carry out VOCs, and then VOCs are trapped in the trap that contains the sorbent such as Tenax [54]. The type of sorbent in the trap can be varied in order, and purge and trap can achieve high selectivity for different biological VOCs. Properly prolonging sampling time could also improve the enrichment effect [53]. This method has been employed for sampling not only VOCs but also semi-VOCs [55].

3.8.2 Solid phase microextraction

Solid phase microextraction (SPME), developed by Arthur and Pawliszyn [56] and Pawliszyn [57], has been considered as one of the best inventions in extraction of volatile organic compounds in the field of sample [53]. SPME integrates the extraction, concentration, and introduction simultaneously, and the use of this method results in reducing preparation time and simultaneously increasing sensitivity over other extractions [53]. Because of all these advantages, SPME could be considered as a simple, efficient and environment friendly sample extraction method, which has been used in the environmental [58], biological [59], pharmaceutical [60], field analyses [61], and fragrance-and-aroma study [62]. Additionally, headspace solid phase microextraction (HS-SPME) has been considered as an appropriate method for sample preparation in the fragrance and aroma analysis [62].

4. Conclusions and future trends

Plants have provided humans with many of their essential needs, including life-saving agents for centuries. As only 1–10% of the available higher plant species have been screened biologically, drug discovery from plants should remain an essential component in the search for new medicines [2], particularly with the development of highly sensitive and versatile analytical methods which include search further for convenient extraction methods in future. Botanical insecticides are also getting a lot of attentions in the integrated pest management to produce healthy fruits, vegetables, and crops [63]. Natural ingredients are also gaining popularity, and the use of plant extracts in cosmetic formulation increases [64]. A cosmetic formulation from natural origin can protect the skin against exogenous or endogenous harmful agents and help to remedy many skin-related diseases [64]. Essential oils have been used for thousands of years, as incense, perfumes, cosmetics, and for their medicinal and culinary applications [64]. This book aimed to cover aforementioned areas and others of applications of plant extracts in depth. It is recommended to conduct international multidisciplinary projects for drug discovery from natural sources, and proper utilization of plants in various areas mentioned in this book is important. Thus, there is a need for cooperative effort among scientists to make use of benefits from these resources.

Author details

Aman Dekebo
Department of Applied Chemistry, Adama Science and Technology University, Adama, Ethiopia

*Address all correspondence to: amandekeb@gmail.com

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Chapter 2

Traditional Chinese Medicine: From Aqueous Extracts to Therapeutic Formulae

Jinfan Wang, Astrid Sasse and Helen Sheridan

Abstract

Traditional Chinese medicine (TCM) is one of the most established systems of medicine in the world. The therapeutic formulae used in TCM are frequently derived from aqueous decoctions of single plants or complex multicomponent formulae. There are aspects of plant cultivation and preparation of decoction pieces that are unique to TCM. These include Daodi cultivation, which is associated with high quality medicinal plant material that is grown in a defined geographical area, and Paozhi processing where the decoction pieces can be treated with excipients and are processed, which may fundamentally change the nature of the chemical metabolites. Therefore, a single plant part, processed in a variety of different ways, can each create a unique medicine. The quality of TCM materials, their safety and therapeutic efficacy are of critical importance. The application of metabolomic and chemometric techniques to these complex and multicomponent medicines is of interest to understand the interrelationships between composition, synergy and therapeutic activity. In this chapter, we present a short history of TCM, detail the role of Daodi and Paozhi in the generation of therapeutic formulae and look at the international practices and methodologies currently in use to ensure their sustainable production, quality, safety and efficacy.

Keywords: chemical fingerprint, chemometrics, cultivation, Chinese herbal medicine, chromatography, chromatographic fingerprinting, Daodi, decoction, metabolomic fingerprint, Paozhi, traditional Chinese medicine, TCM, TCM granules

1. Introduction

Traditional Chinese medicine (TCM) has been used extensively for thousands of years [1] and is the initial medical treatment that the ancient Chinese used to treat wounds and diseases. With the passage of time, Chinese people began to investigate and record the pharmacological activities of the herbs they were using, based on experience. They classified medicinal herbs into five flavours which are pungent, sweet, sour, bitter and salty, forming the earliest system in TCM [2]. As Confucianism and Taoism developed, yin and yang, the five elements (metal, wood, water, fire and earth) and the seven-relation compatibility were introduced and incorporated into TCM. These concepts influenced the development of TCM treatments and therapeutic formulae called Fangji. Fangji are composed of multiple herbs with integrated medical effects and are guided by the concepts underpinning TCM [3].

Herbal materials used in TCM are often extracted with water to make an aqueous extract or decoction. Single herbs, or multiple herbs combined in one formula, can be used to make multicomponent TCMs. Multicomponent therapeutic formulae are the most important and are most commonly used in TCM for clinical applications [4]. The extraction methods employed to produce aqueous decoctions can vary, depending on the different compositions of the formulae. The details of the extraction methods are important as the methodology can impact the chemicals extracted, and hence, the therapeutic effect of the decoction. In this chapter, we will briefly discuss the historical development of TCM and explain some important TCM theories. Understanding the Chinese philosophy of TCM is of great importance, as this will illustrate why TCM is a very different medical system to Western phytomedicine.

As for all herbal medicinal products (HMPs), the therapeutic effects of TCM are influenced by many factors which affect the quality of the starting materials, for example, quality and age of seed stock, climate, soil, humidity, temperature and sunlight. Factors such as storage, contamination and pollutants can also affect the quality of materials [5]. For TCM, there are some unique traditional practices that can determine the therapeutic activity of the materia medica. These notably include specific cultivation, harvesting, fumigation and processing methods of fresh herbal material, which are keys to the quality, efficacy and safety of TCM. Thus, in this chapter, we will discuss two important traditional Chinese medicine terms called Daodi and Paozhi, both are historical words but are still currently used and play important roles in the development of a TCM [6].

'Materia medica' for TCM decoctions is described as Daodi when cultivated under particular conditions in specific geographic regions and processed with specific methods. In TCM, Daodi medicine is recognised as meeting the highest quality standards and denotes superior clinical properties [7]. Modern scientific research supports the fact that Daodi medicinal herbs are more potent than non-Daodi grown samples of the same herb. These observations are further discussed in Section 2.2. The second key term in the production of TCM is Paozhi, which is defined as a group of methods for preparing TCM to generate material with different clinical or therapeutic purposes [8]. Paozhi methods are guided by TCM theory, and their use differentiates TCM from western herbal medicine. In this chapter, we provide specific examples illustrating this difference. For example, the same herb can be processed in different ways, and Shan zha (*Crataegi fructus* 山楂), a fruit, is usually fried. This process results in fruit with the generic term 'Chao'. Different approaches to process the fruit yield different Chao, including Yellow Chao, Charred Chao and Carbonised Chao. The decoctions that result from the different forms of Chao are different in terms of chemical composition, and the resulting decoctions are used to treat different degrees of intestinal disease [9]. Furthermore, processing methods are also very important for the safety and storage of TCM and have a direct impact on the consistency and quality of the Chinese herbal medicine. In general, the variations in quality, safety and efficacy in TCM are the most significant barriers faced by China in gaining access for TCM into European and North-American markets [10].

Currently, sustainability of ecological resources is attracting global attention, especially for medicinal herbal plants. Since they are in large demand in Asian countries and natural products are gaining in popularity in the European and American markets, there is a major challenge relating to sustainable supply of herbal materials [11]. Thus, cultivation is being adopted to solve the problems caused by wild harvesting. In Section 2.3 'Cultivation and wild harvesting' of this chapter, we will discuss the advantages and disadvantages of cultivation for the sustainable supply of quality herbal materials.

Nowadays, as modern analytical techniques become more sensitive and metabolomic methodologies become more refined, chemometric analysis of TCM is used to investigate the relationship between chemical profiles, candidate components and

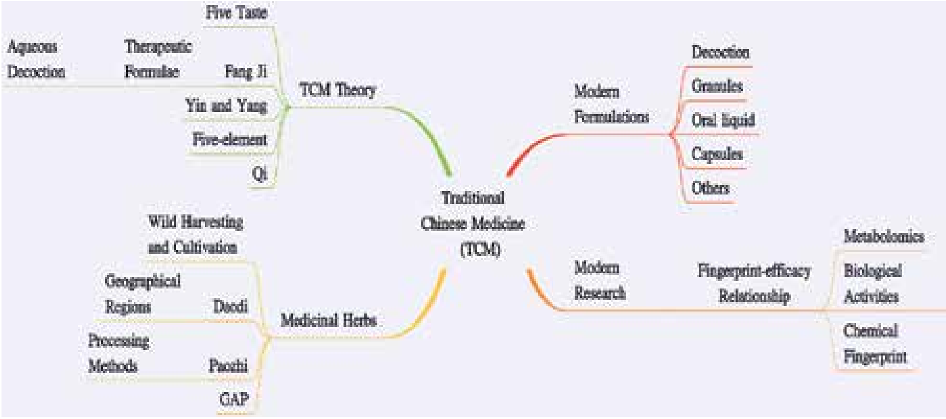

Figure 1.
Main factors that contribute to modern TCM [9, 14, 15].

bioactivities. Multiple methods, such as hyphenated chromatographic and spectroscopic techniques (e.g., liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-nuclear magnetic resonance (LC-NMR)) are applied to determine the chemical fingerprints and to correlate these with bioactivities of TCM. Chemometric techniques advance our understanding of composition and bioactivity of extracts and include, among others, principal component analysis (PCA), linear discriminate analysis (LDA), spectral correlative chromatography (SCC) and information theory (IT) [12].

The guidance of TCM theory for the generation of Paozhi and therapeutic formulae impacts the chemical composition and final therapeutic effects of medicinal herbs. In contrast, phytomedicine focuses on identification and isolation of individual chemical components, lacking the characteristics of Fangji in traditional Chinese medicine theory, where herbal formulae are organised using the Jun-Chen-Zuo-Shi system. Combining the pharmacological analysis of multiple biomarkers with chemical fingerprint analysis can help to provide an understanding of how the therapeutic effect of herbal formulae is produced [3].

More recently, modern formulations of traditional TCM decoctions have come on the market. These involve the formation of granulated material, by combining decoctions with excipients and subjecting them to spray drying and granulation to create stable products. Such dried decoction material can also be incorporated into capsules. Modernised TCM formulations are more easily transported and stored and can have a long shelf life than the original herbal material. Some commonly-used TCMs have been formulated in such a way [13]. However, the efficacy of these emerging, modernised TCM formulations has not been fully evaluated by international researchers. In addition, solving the efficacy equivalence between decoction pieces and new formulations is an important problem for the modernisation of TCM [14].

Therefore, TCM is the final product of several complex factors, that is, TCM theory, medicinal herbs, modern formulations and modern research as detailed in **Figure 1**.

2. From aqueous extracts to therapeutic formulae

2.1 History of traditional Chinese medicine

Astronomy, arithmetic, agronomy and traditional medicine were the most advanced areas in of science ancient China. Traditional Chinese medicine (TCM) is one of the oldest medical treatment systems worldwide, with thousands of years' history. It encompasses herbal medicine, animal medicine, acupuncture, moxibustion [a TCM therapy which uses dried Mugwort (or other herbs) burnt on or near the skin to promote healing], therapeutic massage, food therapy and physical exercise [16]. Today, TCM is still used in China and other Asian countries, such as Japan, Korea, Singapore and Malaysia. TCM is one of the most significant alternative treatment systems known and is now increasingly accepted and more widely used by the Western world. In the 2009 Health Department of Taiwan report, it is stated that approximately 28% of the population over 15 years old have used TCM treatment at least once [17]. One key example is the treatment of Alzheimer's disease using TCM such as Ji-Sheng-Shen-Qi-Wan, Ma-Zi-Ren-Wan and Tian-Wang-Bu-Xin-Dan. These medicines are commonly used to treat mental disorders and nervous system diseases, including Alzheimer's disease [18]. A clinical trial on the pharmacological activities of Tian-Wang-Bu-Xin-Dan shows that it can reduce the level of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) from elderly dementia patients with sleep disorders, thus demonstrating a measurable effect on biochemical markers [19].

In ancient China, the effect of many medical materials was identified by tasting, and the taste of herbs was classified into five flavours (pungent, sweet, sour, bitter and salty), with each flavour representing different drug properties [20]. Over time, people learned more about the healing properties of herbs and learned how to use them for treatment of different diseases; for instance, *Coptis chinensis* (Huang lian 黄连), which is known to have a bitter taste, was used to treat diarrhoea [21]. In Yan Di's legend, Shen Nong organised the tasting of hundreds of herbs and often recorded poisonous effects. Thus, great knowledge of activity and toxicity was accumulated, and following many trials, the applications for efficient medicines were recorded and summarised, resulting in the earliest recordings of TCM (**Figure 2**). Two of the earliest Chinese medicine books, the 'Huang-Di-Nei-Jing' (770–221 BC) and the 'hen-Nong-Ben-Cao-Jing' (25–220 AD) [22] had great influence on the TCM development in the long history of China [23].

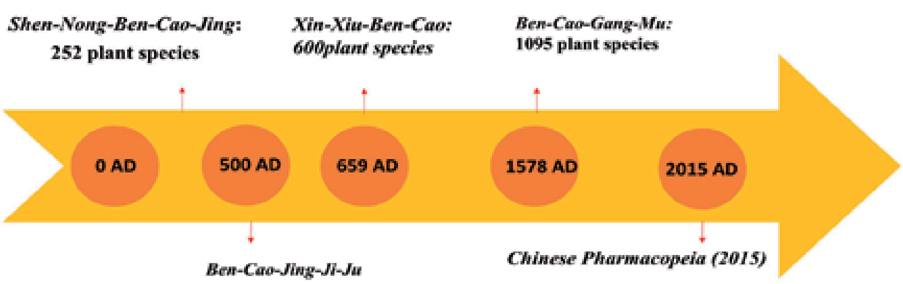

Figure 2.
Timelines of some ancient Chinese medicine books [22].

Decoction of herbs, the most important preparation method of Chinese medicine, was first invented and further developed between 2000 and 474 BC. The medical ingredients were extracted by boiling in water or alcohol, with special preparation times that depend on the properties of the ingredients. Following this, the decoction was filtered, and the resulting liquid was taken by patients. In modern China, the method of decoction is still the most commonly used process in TCM [24].

In TCM, most of the disease diagnosis and principles of medical application are based on a particular Chinese philosophy that is aligned with Confucianism and Taoism [25]. The theory of TCM refers to Yin and Yang, the five elements, zangfu, channels-collaterals, qi, blood, body fluids, methods of diagnosis, differentiation

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of symptom-complexes, etc. Yin and Yang are opposite and complementary sides of the nature in the universe, and, according to Chinese philosophy, everything could be described by Yin and Yang (**Figure 3**) [16]. In TCM, Yin refers to the material aspects of the organism, and Yang refers to functions. It is the interpretation that the disease is caused by the imbalance of Yin and Yang in the human body. The rationale of Chinese medicine is to bring Yin and Yang back into balance, which results in overall health and cure versus the disease [24].

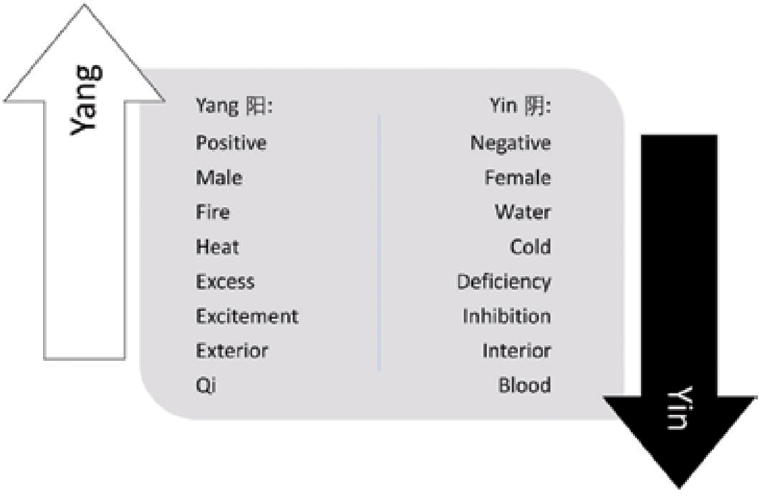

Figure 3.
Yin and Yang theory [26].

In addition, in ancient Chinese theory, everything in the universe consists of five elements (metal, wood, water, fire and earth) (**Figure 4**). All organs and tissues were assigned to different elements, which are differentiated by their properties [20]. This is the special Chinese system theory that forms a basis for TCM. Chinese

Figure 4.
The principle of the five elements system (Wu xing 五行) [27].

medicine is very different from Western medicine, and the methodology of disease treatment cannot be explained in the same way as in modern medicine, as it is a type of treatment that is based on experience and a special philosophy [25].

After the 1950s, advanced research into TCM began, which was aimed at meeting the needs of the growing Chinese population and also at reaching the standards of safety, efficacy and quality of Western medicine [28]. In the 1970s, Youyou Tu was inspired by a book about TCM, the 'Zhou-Hou-Bei-Ji-Fang' (317–420 AD), in which *Artemisia carvifolia* (Qing hao 青蒿) was described as being extracted with water to produce a decoction, and this was used to treat malaria symptoms. This led her to isolate artemisinin from the Qing hao plant. Artemisinin was successfully tested for the treatment of epidemic diseases including malaria in China, which ultimately led to Youyou Tu to being awarded the Nobel Prize in 2015 [29].

With the passage of time, TCM was also delivered in increasingly diverse formulations, other than decoctions, such as wines, pills and plasters. Currently, modernised formulae such as granules, oral liquids, capsules, dissolved medicines and ointments are coming onto the market. These modern formulations are produced by a small number, currently six, of State approved pharmaceutical industries [30]. China introduced the concept of 'internationalization of TCM' in 1996, to address sustainable production and to promote export and international trading, and as a result, the quality, safety and regulatory requirements for TCM have gained increasing international attention and have promoted the formation of international consortia (e.g., GP-TCM; http://www.gp-tcm.org/) working to this end [31].

2.2 Daodi herbal medicine

Daodi is a term unique to TCM and is reserved for medicinal plants cultivated in a specific geographical area with specified natural conditions and being harvested and processed following standards. 'Dao' refers to the measurement unit of districts in ancient China, and 'di' refers to earth, land or soil. It is stated in an ancient materia medica 'Xin-Xiu-Ben-Cao' that the medical efficacy will be different if the medicinal material is not grown in its native environment [32]. Daodi medical material has been regarded as superior medicine for centuries and is recognised as such by today's TCM pharmaceutical industry (**Figure 5**). From the author's observation of Daodi *Houttuynia cordata* and non-Daodi *Houttuynia cordata* plant material, the Daodi material is more vibrant and less dense. In the Chinese Pharmacopoeia (2015), 284 different kinds of TCM plant materials are recognised to have Daodi specifications from a total of 584 commonly used medicinal plants [7].

TCM knowledge and expertise have grown over millennia of clinical experience, resulting in the discovery and understanding of the differing properties of herbal material, which depend on their quality, and often are impacted by the source. The earliest description specifying certain regions for medicinal plant production can be retraced to the late Eastern Han Dynasty (25–220 BC) and was recorded in the 'Shen-Nong-Ben-Jing' (**Figure 6**). The book states that the properties of medicinal plants vary from region to region [32]. In the Ming Dynasty (1368–1644 BC), the Daodi concept was initially described in the Materia medica 'Ben-Cao-Pin-Hui-Jing-Yao' [33].

At that time, the detail relating to the production region was recorded by very simple and basic descriptions, for example, 'grows on mountain valleys, river valleys or marshes'. With increased experience and accumulated knowledge, ancient people started to name some medicinal plants by the names of regions, for instance, *Radix Morindae officinalis* (Ba ji tian 巴戟天) or *Crotonis fructus* (Ba dou 巴豆) [36]. 'Ba' was the name of a Sichuan province, hence the Daodi region of Ba dou and Ba ji tian is Sichuan. In 420 BC, it was stated clearly in the book 'Ben-Cao-Jing-Ji-Zhu' that the term 'Zui jia', which means 'the best' in Chinese, should be used to describe

Figure 5.
Commercial TCM products: (A) Houttuynia cordata (Yu xing cao 鱼腥草). (B) Radix Dioscoreae oppositae (Shan yao 山药). (C) Daodi Houttuynia cordata decoction pieces. (D) Non-Daodi Houttuynia cordata decoction pieces.

the appearance and quality of plants grown in specific regions. Later, in the Tang dynasty, the term 'King of Medicine' was applied to describe the best medicine which is recorded in the book 'Bei-Ji-Qian-Jin-Yao-Fang (Qian-Jin-Fang)', and shortly after, Sun simiao in his book entitled 'Xin-Xiu-Ben-Cao' created the term Daodi [32].

In ancient times, Chinese people found that the taste of mandarin which was produced south of Huai River were sweet; however, mandarins that were produced in the north of Huai River were bitter. Although the leaves and appearance were similar, the flavour was completely different. Thus, ancient Chinese people were aware of the fact that geographical changes may influence the activity of herbal material used in TCM [32]. The characteristics of plants are determined by genetics; however, the diverse landforms and weather in China resulted in different ecosystems, which contributed to a variety of botanical germ plasm origins [37]. Additionally, specific ecological conditions such as topography, soil, climate, humidity and light also influenced the number of secondary metabolites of

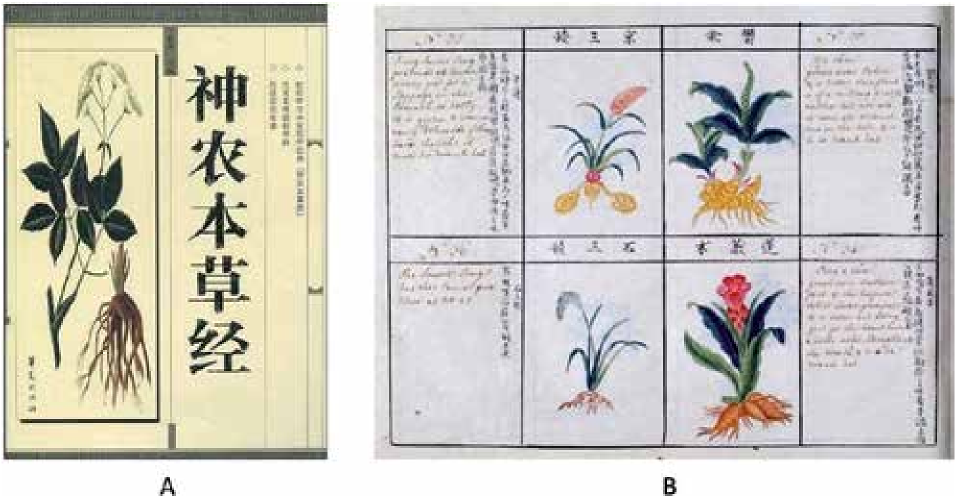

Figure 6.
Ancient TCM texts: (A) 'Shen-Nong-Ben-Cao-Jing'. (B) 'Ben-Cao-Gang-Mu' [34, 35].

plants, which resulted in different bioactivities of a particular herbal material. Therefore, the combination of the geographical location and the specific germ plasm resulted in the superior quality of the Daodi medical material [38].

In the first modern monograph of Daodi medicinal material in the Chinese Pharmacopoeia (1953), 159 different medicinal materials are classified into 8 categories according to the various production regions in China (**Figure 7**): Chuan (Sichuan), Guang (Guang dong/Guang xi), Yun (Yun nan), Gui (Gui zhou), Nan (Southern China), Bei (Northern China), Zhe (Zhe jiang) and Huai (He nan). Interesting examples include *Liguisticum chuanxiong* Hort. (Chuan xiong 川芎), which belongs to Chuan Daodi medica material, because the Sichuan province is the native region for cultivation and is known to have the largest output and longest history of medical application of Chuan xiong. *Liguisticum chuanxiong* Hort. cv. *Fuxiong* (Fu xiong 付芎) is produced in the Jiangxi province and has large and fleshy rhizomes similar to Daodi Chuan Xiong; however, it has been shown that it contained less essential oil by chemical analysis, which resulted in different bioactivities [39]. Ginseng is one of the most famous and expensive TCM plant materials, and the Daodi production region of ginseng is in North-Eastern China. In 1958, ginseng was cultivated on the Hailan island in Southern China, which resulted in large roots which were lacking bioactive constituents [32]. In the past 10 years, chemical constituents of volatile oil in the rhizomes and radices of *Notopterygium incisum* Ting (Qiang huo 羌活) from different regions were investigated by GC. In the Sichuan Daodi sample, 769 compounds were identified and quantified and showed significant differences in the chemical composition of samples that were produced in other non-Daodi geographical regions [40].

Nowadays, with the increased need for herbal medicines for health purposes, including food supplements, nutraceuticals and skincare products, it is recognised that the Daodi region cannot produce sufficient plant materials to meet the market demand [41]. Since 2002, the Chinese government is emphasising Good Agricultural Practice (GAP) for the cultivation of medicinal materials, which is aimed at expanding the production regions. According to the Good practice of TCM report of the European commission in 2012, Daodi medicinal herbs and also new Daodi medicines will be allowed to emerge [42]. For instance, Daodi *Radix Salviae miltiorrhizae* (Dan shen 丹参) that was cultivated exclusively in Sichuan, is now produced on large-scale standardised plantations in the non-Daodi region Shanxi province by modern TCM pharmaceutical companies [32]. The Tasly pharmaceutical company

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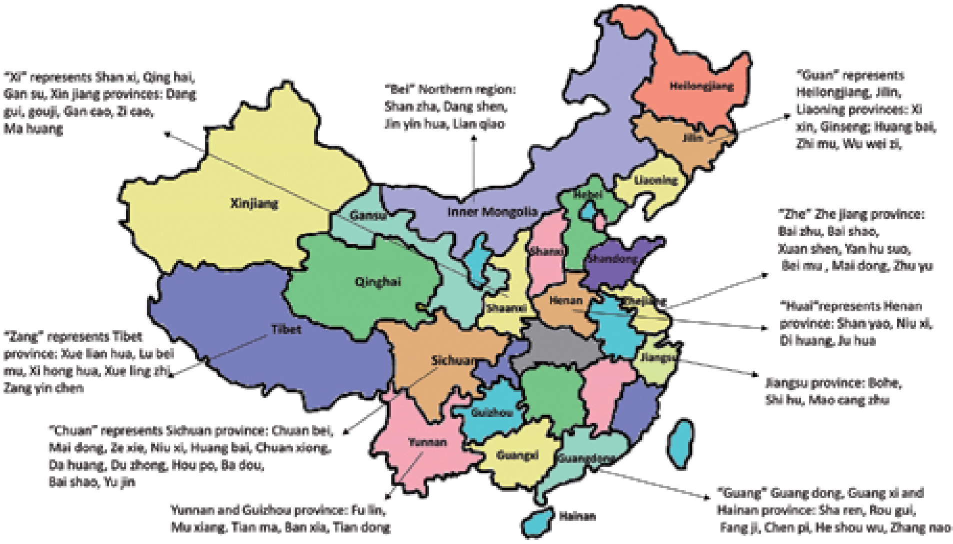

Figure 7.
Map of Daodi medicines produced in 10 regions in China [24].

was the first, approved by GAP, in China for the large-scale standardised plantation [43]. Goji (fruits of *Lycium barbarum* L. and *Lycium chinense* Mill), which has been applied in TCM treatment for millennia to treat imbalance of yin, is now experiencing an increased demand as it is also seen as 'super food' and recommended as potent antioxidant in the Western world [44, 45]. The Ningxia province is recognised as the Daodi region for Goji, but the increasing market led to new cultivations in other regions such as in the Hubei, Qinghai and Xinjiang province, where the geographical and climatic conditions are different to the Daodi region. Goji produced from those regions also has very high quality, but is different in the number of metabolites, such as polysaccharides, flavonoids, betaine and carotenoids [46] Therefore, it is still challenging to achieve the aim of GAP. In Yao's group, the antioxidant activities of Daodi Goji and non-Daodi Goji were studied, and the IC50 values of the radical-scavenging effect for both Goji treatment groups were determined to be even higher than the positive control group which was treated by Quercetin. Notably, the Daodi Goji shows the highest anti-oxidant activities of all tested materials in this study [44].

Daodi medicinal material plays an essential role in TCM treatment, but its superior quality has not yet been adequately explained by modern science [47]. Comparisons between Daodi and non-Daodi materials have been studied mainly in terms of bioactive ingredients or composition, soil properties, the geological background system (GBS) and some identification methodologies to assess Daodi attributes, which might be closely related to bioactive ingredient production [33]. However, the superior quality, safety and efficacy of Daodi products still remain to be investigated. Current research of Daodi products focuses on the identification and authentication of species in the Daodi region, establishment and implementation of the commercial specification criteria and standardisation of plantation and processing [48]. Therefore, there is a need for further research in TCM to establish many factors that influence the potency of Daodi medical material.

2.3 Cultivation versus wild harvesting

China is reported to have the largest number of medicinal herbs, with approximately 11,146 being used in 2016 [49]. TCM is based on wild medicinal herbs resources, and it was stated in 2015 that they account for approximately 80% of the total Chinese herbs resources [11]. To meet the increasingly higher demands from the domestic and global market and also to achieve the sustainable utilisation of medicinal herbs, some scale-up cultivation projects for TCM herbs have been established [49]. Moreover, natural foresting, which is closer to wild harvesting but different to conservative artificial cultivation, is recommended to be applied for production of some medicinal herbs, such as *Fritillaria cirrhosa* (Chuan bei mu 川贝母), *Radix Ginseng* (Ren shen 人参) and Huang lian, *but* the cost is much higher than that of cultivation [50]. It is anticipated that cultivation of medicinal herbs will satisfy the market demand and reduce the ecological pressure caused by wild harvesting. Outcomes for cultivation show that it is an approach suited to some herbal material, and the appearance and efficacy are very close to herbs harvested wildly. However, high upfront costs can be associated with the construction of the special environment needed for mass cultivation, but the economic and ecological benefits of cultivation versus wild harvesting are recognised [51].

However, the consistent quality of herbs from artificial cultivation and wild harvesting is still a challenge for most TCM herbs, because quality, safety and efficacy of medicinal herbs can be impacted by variations in cultivation. For example, the active components of *Ranunculus ternatus* are polysaccharides, and the concentration of which varies significantly between samples collected by natural fostering and wild collection, 10 and 14%, respectively [52]. To solve the problems of artificial cultivation, standard operating procedures, which include germ plasm selection, breeding, fertilisation, irrigation, pest control and limitation of toxic substances, are required to improve the quality of TCM close to that of the wild harvested herbs [53]. In the 'Guidelines for Good Agricultural Practice of Chinese Crude Drug', the GAP standards for four medicinal herbs, *Ginseng*, goji, *Radix glycyrrhizae* (Gan cao 甘草) and yu xing cao are described in detail [54].

Cultivation and wild harvesting have advantages and disadvantages. For cultivation, soil fertility has a significant impact on the yield of the major chemical components in herbal materials, and the lack of standards for soil fertility will result in unsure biomass and quality of medicinal herb production. The standards for soil fertility should include soil texture, density, moisture content, soil acidity, nitrogen and phosphorus content and microorganisms. For wild harvesting, the quality of medicinal herbs is determined by factors such as identification, time of harvesting, harvesting methods and transportation. Harvesting methods should avoid damage to harvested material and to surrounding herbs. The ideal quantity of harvested herbal material should be scientifically considered and balanced, to allow for sustainability. The period of harvesting is important as the effective chemical contents of herbs vary seasonally and climatically, which will impact on biological activity of the decoctions derived from the herbal material [50, 55]. The effects of global warming on plant cultivation, sustainability and quality will have to be considered in the coming years, a topic under investigation at Kew gardens (https://www.kew.org/science/projects/chinese-medicinal-plants-and-their-materia-medica).

Wild harvesting can be considered 'treasure from the nature', as the material comes from the natural resource without the addition of pesticides. Furthermore, for most of medicinal herbs, wild harvested material is superior, with resultant decoctions being more potent. However, overloaded harvesting causes an imbalance in the ecological environment and threatens the quantities of wild resource of especially rare or endangered species. *Codonopsis pilosula* (Dang shen 党参), Dan shen, *Radix isatidis seu Baphcacanthi* (Ban lan gen 板蓝根), ginseng and *Coptis chinensis* (Huang lian 黄连) are some of the key TCM herbs cultivated in different regions in China [50]. Without doubt, the management policy and practice of wild harvesting of natural resources require further development, if goals of sustainable supply of this resource are to be achieved.

In conclusion, cultivation of medicinal plant material reduces the ecological impact caused by extensive wild-harvesting and can also protect the standardised genotypes of species, contributing to the sustainable production and utilisation of Chinese medicinal herbs. Cultivation also has an economic benefit, with new industries and employment models emerging, and guaranteeing the supply for the expanding and substantial Chinese and/or global markets. However, one negative impact is that the natural diversity of the gene pool for wild resources is reducing due to the standardisation of mass cultivation practices [49]. A reasonable balance of cultivation, wild harvesting and natural foresting will ultimately be the best model and will contribute to the sustainability of TCM herb resources (**Figure 8**).

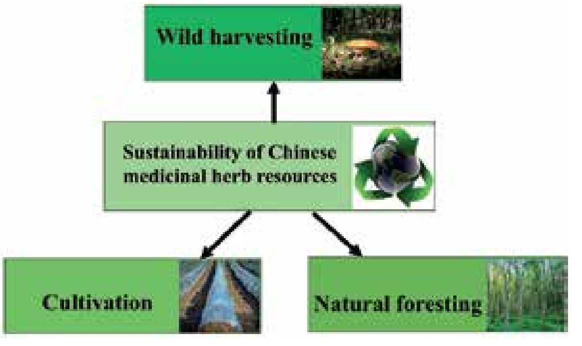

Figure 8.
Wild harvesting, natural foresting and cultivation contribute to the sustainability of Chinese medicinal herb resources [56–59].

2.4 Paozhi

Paozhi is a unique traditional processing technique, which differentiates TCM from Western herbal medicine. The processed products following Paozhi are known as decoction pieces [60]. With the guidance of TCM theory and according to the properties of medicinal plants, the raw herbal medicine is processed to suit the TCM therapeutic purpose [8]. In TCM theory, it is the belief that disease is caused by the imbalance of Yin and Yang, and ancient Chinese people believe that the processing procedure could adjust the 'Qi' (heat, warm, cold and cool qualities) of raw herbal medicines [61], to equilibrate the balance of Yin and Yang in the human body.

Processing methods are usually different for different medicinal plants, and this is due to the nature of the material, for example, *radix* (root), *rhizoma* (rhizome or lateral root), *herba* (herb, whole plant) and *flos* (*flower*) and the purpose for their clinical application. The main aim of Paozhi is to enhance the efficacy and reduce toxicity [60]. For instance, *Radix Aconiti kusnezofii* (Cao Wu tou 草乌头) is processed to reduce toxicity for safe use by steaming or boiling with *Radix glycyrrhizae* (Gan cao 甘草) and *Semen Sojae hispidae* (Hei dou 黑豆). Paozhi is also applied to remove disagreeable odours and flavours of medicinal plants, for example, *Herba* sargassii (Hai zao 海藻) is processed by a clear water rinse. Most herbs are sliced, shaved, or chopped, which is favourable for preparation, storage and pharmaceutical production [62].

The processing mechanism of Paozhi can be explained by a direct reduction of toxic contents and constituents, structural transformation of constituents and influence of excipients (**Figure 9**). Most Chinese Herbal Medicines (CHMs) need to be processed prior to use in clinical therapy. In the Chinese Pharmacopoeia (2015), decoction pieces and related processing methods are laid out in specific chapters of CHM, and some decoction pieces are recorded with specific quality control standards and indications, such as *Radix Astragali membranacei* (Huang qi 黄芪), which helps strengthening the immune response [63].

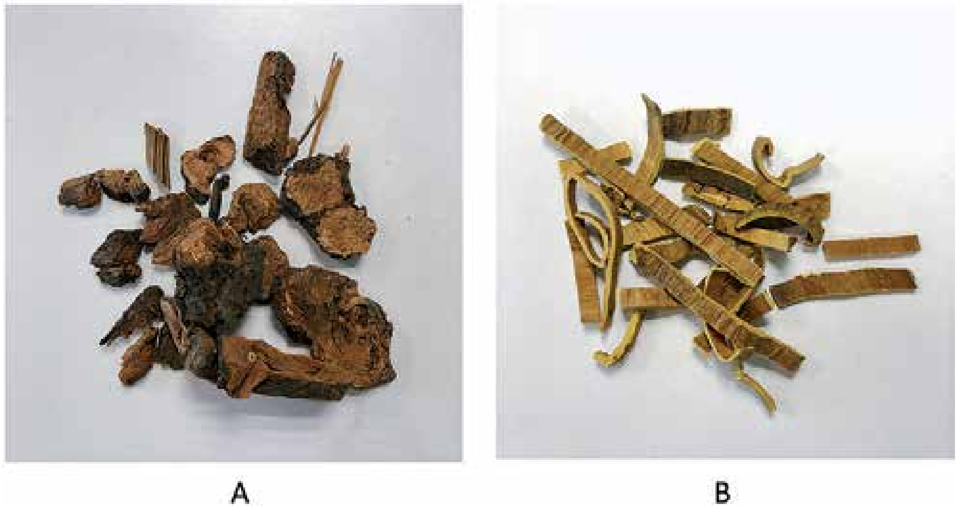

Figure 9.
Paozhi processed root and bark samples. (A) Radix sanguisorba Officinalis L. (Di yu 地榆). (B) Phellodendron chinese Schneid (Huang bai 黄柏).

The earliest description of Paozhi can be traced back to 200 BC and classic methods, including burning, stewing and soaking with wine and vinegar, were recorded in 'Wu-Shi-Er-Bing-Fang' [60]. In the Ming Dynasty (1368–1644 AD), the processing methods according to Paozhi flourished and became more refined and creative as a variety of additional excipients was introduced in herbal medicine processing [64]. Later, in the first monograph of processing methods 'Lei-Gong-Pao-Zhi-Lun' (around the fifth century), the Paozhi processing methods of 268 TCM materials were recorded, of these, 178 medicines were prepared with excipients (**Table 1**). These comply with the theory of 'seven-relation compatibility' in the TCM theory system [65]. When two or more herbal TCMs are combined in one formula, the 'seven-relation compatibility' is applied, which is very important for Paozhi. For example, 'xiang wei' means one herbal medicine can reduce the toxicity of another medicine, thus the toxic herbal medicine *Rhizoma arisaematis Preparatum* (Tian nan xing 天南星) is traditionally processed with ginger to reduce its toxicity for clinical safety. Studies of chemical changes in processed Tian nan xing showed that levels of calcium oxalate, which is recognised as a toxic substance, were reduced by 50% compared to the raw herbal material [66]. In 1662, processing methods of 439 Chinese medicines were described in the materia medica 'Pao-Zhi-Da-Fa'. In the Qing dynasty, the monograph of Paozhi method was recorded in the book 'Xin-Shi-Zhi-Nan', which encompassed many classic methods from the long history of processing herbal medicines [67].

Processing is the way that natural medicines are transformed into TCM by physical or chemical methods. Processing also increases efficacy and/or reduces inherent toxicity. The main processing methods encompass a variety of techniques such as cleaning, cutting, crushing, roasting, boiling, baking and stir-frying, with or without liquid/solid excipients [8]. 'Chao' means stir-frying in Chinese. Clean and cut materials are stir-fried until a colour change to yellow is observed, or they

Paozhi method	English translation	Excipient added	Examples of processed medicine
"Chao"	Stir-frying	-	"Chao" *Crataegi Fructus* (Shan zha 山楂)
"Zhi"	Stir-frying with liquid excipients	Yellow-rice wine	"Zhi" *Radix Et Rhizoma Rhei* (Da huang 大黄)
"Zheng"	Steaming	Vinegar	"Zheng" *Schisandrae Chinensis Fructus* (Wu wei zi 五味子)

Table 1.
Commonly used Paozhi methods with examples of medicines [61].

may be either charred or 'carbonised'. Carbonised in 'chao' refers to decoction pieces that are black on the outside and brown on the inside. These three stages of processing represent a different degree of 'Chao' and infer a different medical efficacy. For example, *Crataegi fructus* (Shan zha 山楂) is used for enhancing digestion, which can be stir-fried into these three stages. Yellow 'Chao' Shan zha is usually used to treat indigestion and charred 'Chao' Shan zha is used for diarrhoea, whereas, carbonised 'Chao' Shan zha is used for gastrointestinal haemorrhage (**Figure 10**).

'Zhi' means stir-frying with liquid excipients. Clean and cut material is stir-fried with a liquid, such as vinegar, salty water, honey, yellow rice wine or ginger sauce, which allows the liquid to be absorbed by the medical material. For instance, *Radix Angelicae sinensis* (Dang gui 当归) is well-known to invigorate blood circulation in the human body, and this effect is enhanced when Dang gui is stir-fried with yellow rice wine. The chemical analysis of processed *Radix Angelicae sinensis* shows significant variation of the amount of ferulic acid and Z-ligustilide, and these chemicals are proved to have an effect in anti-platelet aggregation [68].

'Zheng' means steaming, which can alter the properties of a variety of herbal medicines. For instance, *Radix polygoni Multiflora* (He shou wu 何首乌) is soaked with black bean sauce and then steamed in a non-ferrous container until the colour is changed into brown, then it is sliced into pieces and dried in the sun. The raw He shou wu is used for its anti-malarial properties, while 'Zhi' He shou wu has different therapeutic indications including improving the kidney function, hair blackening and strengthening of

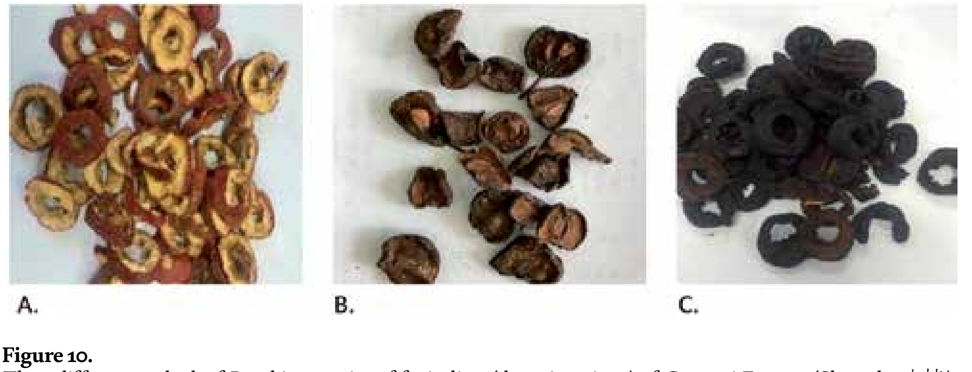

Figure 10.
Three different methods of Paozhi processing of fruit slices (decoction pieces) of Crataegi Fructus (Shan zha 山楂): (A) Yellow 'Chao' (stir-fried until a colour change to yellow is observed). (B) Charred 'Chao' (stir-fried until a colour change to brown in achieved). (C) Carbonised 'Chao' (stir-fried until colour change to black on the outside and brown on the inside is attained).

bones. Pharmacokinetic studies of typical constituents from processed *Radix polygoni Multiflora* by LC-ESI-MS/MS indicate that the processing method can improve the bioavailability of garlic acid and decrease the absorption of 2,3,5,4′-tetrahydroxystilbene-2-O-β-d-glucoside (PM-SG), resveratrol and emodin in rat plasma [69].

The different processing methods have varying influences on medicinal herbs, and this contributes to diverse medical activities and reduced toxicity. For example, *Radix et Rhizoma rhei* (Da huang 大黄) is commonly processed by four classical methods which are recorded in the Chinese Pharmacopoeia (2015). This encompasses 'Sheng' Da huang (dried raw material), 'Zhi' Da huang (stir-frying with wine), 'Zheng' Da huang (steaming with wine) and carbonised 'Chao' Da huang (stir-frying till carbonised) (**Figure 11**). Each of these four processed Da huang products has different medical effects. 'Sheng' Da huang is often used for constipation in clinical practice, and 'Zhi' Da huang is effective for hematemesis, headache and toothache. Chemically, this may be explained by decomposition of conjugated anthraquinones into the corresponding free anthraquinones. 'Zhi' Da huang has an effect on constipation; however, the effect is weaker than for 'Sheng' Da huang, which enhances blood circulation, and this may result from decreased contents of tannins. Carbonised 'Chao' Da huang has an effect on haemostasis for hematochezia and no effect on blood circulation [70].

Nowadays, the difference between processed and non-processed medicines is studied by modern scientific analytic methods. For example, *Radix aconiti* (Chuan wu 川乌) is an essential herbal medicine, which has a long history of application in TCM as it has a wide range of medical effects, such as anti-inflammatory and analgesic properties. However, the non-processed Chuan wu is very toxic and induces remarkable neuro- and cardiotoxicity. Chuan wu is processed by boiling and drying [61]. The comprehensive metabolomic characteristics of non-processed and processed samples were investigated by a LC-MS method, which identified specific metabolite changes. In this study, 22 key biomarkers were related to detoxification by processing. Diester diterpene alkaloids (DDA)

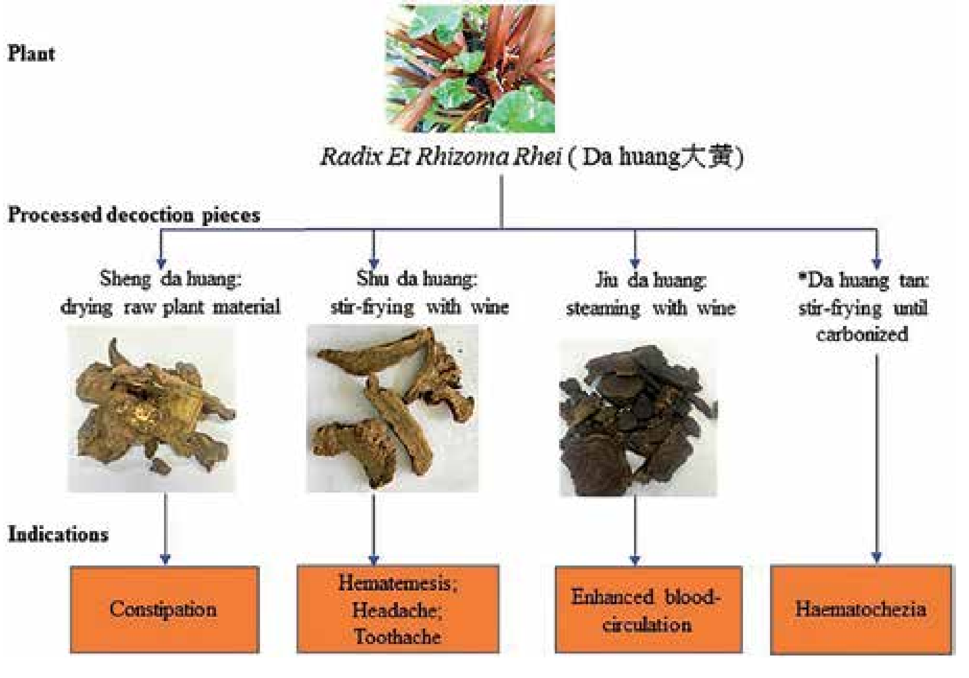

Figure 11.
*Examples of Da huang processed by different methods and applied for different indications. *Da huang tan is rarely used, so an image is unavailable.*

and toxic monoester diterpene alkaloids (MDA) were identified to be the main components which were reduced by this processing method for Chuan wu [71]. The contents of magnolo and honokiol from *Cortex magnoliae Officinalis* (Hou pu 厚朴) were reduced by 14 and 40%, respectively, after processing by stir-frying with ginger, and the new component gingerol was formed [40, 72]. The solubility of active constituents can be improved to enhance the efficacy by processing, for instance, Huang lian, traditionally used for toothache, liver disease or inflammation and can be processed by stir-frying with wine, vinegar, salt, bile, or *Fructus evodiae* (Wu zhu yu 吴茱萸). Chemical analysis of processed Huang lian suggests that the contents of alkaloids are different, depending on the processing method but all are higher than non-processed material, and stir-frying with wine leads to the highest contents [73].

Triterpene saponins were found to be the main bioactive constituents of Ginseng, which has anti-oxidant, anti-diabetic, immunomodulatory, anti-inflammatory and anti-cancer activities [74]. After processing by steaming and drying, the chemical profiles are remarkably different between processed and non-processed Ginseng. The structural transformation of ginsenosides in processed Ginseng, which proved to have more potent bioactivities, contributes to its enhanced efficacy [61]. Solubility is influenced by processing, and by the addition of excipients, such as wine, honey, vinegar, salt water and ginger juice. Excipients may react with constituents in herbal medicines and may have effects on active constituents, for example, it was reported that ginger juice has anti-inflammatory activity itself which contributes to the detoxification effect of processed *Rhizoma pinelliae Tematae* (Ban xia 半夏). Therefore, processing can result in reducing amounts of toxic constituents, structural transformation and solubility improvement of active constituents.

In addition to daodi, Paozhi also plays an important role in TCM in influencing the quality, safety and efficacy of medical materials. There is a continuing need for multidisciplinary research to fully evaluate the effects of these practices, in order to increase the understanding of their impact on the chemical fingerprints of therapeutic decoctions and on the mechanism of action of resultant TCMs [60]. In different regions of China, the processing methods of TCM vary; for instance, white rice wine is commonly used in the Hunan province, but yellow rice wine is used in Fujian, Anhui and Guangxi province. Today, there is still no consistency of processing practice across China. Hong Kong is the international centre of TCM, and most of the TCM decoction pieces in the western world are imported from Hong Kong. It is reported that 66% of 365 kinds of TCM decoction pieces of commonly used TCM are processed locally in Hong Kong, where the processing methods are different to those in the mainland of China. Such differences may result in a Butterfly effect as eluded by Sheridan et al. [8] and may result in very different therapeutic effects associated with the final TCM. Consistent and standardised processing methods are required for the global development of TCM [75].

In recent years, the study of Paozhi is focused on understanding and validating the traditional aspect of processing. The chemical profiles of processed herbal medicine are investigated by NMR, GC and LC-MS analysis methods, and metabolomic profiles are studied with chemical markers. In addition, toxicity or side-effects can result from improper processing methods, and therefore, a standardisation of processing methods for TCM is essential. It seems appropriate that in the modernisation of TCM, significant chemical contents and pharmacological indications should be applied as evaluation markers, a quality control standard should be established to reinforce the GMP of processed products, to optimise the process procedure and standardise the quality, safety and efficacy of decoction pieces.

2.5 Seven-relation compatibility

Multiple herbs can be combined in TCM, with up to 20 herbs in one formula, yielding very complex decoctions. Such a complex formula is called Fangji in Chinese, and it is adjusted for each individual patient under the guidance of TCM theory. At times, single herbs are used to prepare decoctions for treatment, and for instance, *Radix Astragali membranacei* (Huang qi 黄芪) is used on its own to treat lung disease. The combination of herbs within one formula is under the guidance of the 'seven-relation compatibility' (**Table 2**), which includes the following: 'mutual accentuation', 'mutual enhancement', 'mutual counteraction', 'mutual suppression', 'mutual antagonism' and 'mutual incompatibility' between two herbs. In addition, single herbs can be used under the 'seven-relation compatibility' in an 'individual application' for the treatment of certain diseases [76].

Chinese Name	English Translation	Explanation	Example of Application
Xiang xu	"Mutual accentuation"	Two medicinal herbs which have similar effects are applied together to achieve better efficacy.	Ginseng is combined with *Radix Astragali Membranacei* (Huang qi 黄芪) to improve the "qi" and the therapeutic efficacy.
Xiang shi	"Mutual enhancement"	Two medicinal herbs which have different effects but can be combined to enhance the effect of the main medicinal herb.	*Poria Cocos Wolf* (Fu ling 茯苓) is applied with Huang qi to improve the efficacy of Huang qi.
Xiang wei	"Mutual counteraction"	Two medicinal herbs applied together because one of them can reduce the toxicity of the another.	Ginger can reduce the neurotoxicity of *Rhizoma Pinelliae Tematae's* (Ban xia 半夏).
Xiang sha	"Mutual suppression"	Two medicinal herbs are applied together because one of them can eliminate the toxicity of the another one.	Ginger can suppress numbing and paralytic effects on the respiratory system of *Rhizome Arisaematis Preparotum* (Tian nan xing 天南星).
Xiang wu	"Mutual antagonism"	The therapeutic effects will be eliminated if the two medicinal herbs are applied together.	*Semen Raphani* (Lai fu zi 莱菔子) decreases Ginseng's efficacy.
Xiang fan	"Mutual incompatibility"	Two herbs which can be safely used separately will exert toxicity, if they are applied together.	When Wu tou is applied with *Rhizoma Pinelliae Tematae* (Ban xia 半夏), they exert toxicity in the CNS system.
Dan xing	"Individual application"	One medicinal herb can be applied on its own.	*Radix Astragali Membranacei* (Huang qi 黄芪) is used to treat lung disease.

Table 2.
Explanation and application of 'seven-relation compatibility' [24].

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2.6 Fangji

Fangji is defined as formulae composed of multiple herbs with integrated medical effects, and it is guided by 'Jun Chen Zuo Shi' theory [3]. One TCM formula generally consists of four different kinds of herbal medicine, which is called 'jun', 'chen', 'zuo' and 'shi' (**Figure 12**). Each of them plays a different role within the formula. All of these medicinal herbs work harmoniously together to achieve therapeutic effects and bring the balance of Yin and Yang back to the human body [77]. In the TCM formula theory system, 'jun' means the 'master' and provides the principal therapeutic effect for the disease; and 'chen' represents the 'advisor' which functions as the second principal medicinal component, supporting the medical efficacy of the 'jun' medicine. 'Zuo' represents the 'soldier' and is applied to treat associated symptoms or reduce toxicity of the 'jun' medicine. 'Shi' represents the 'guide' which can direct other medicines to the diseased organ or contribute to the harmony of all herbs in the formula [3]. For

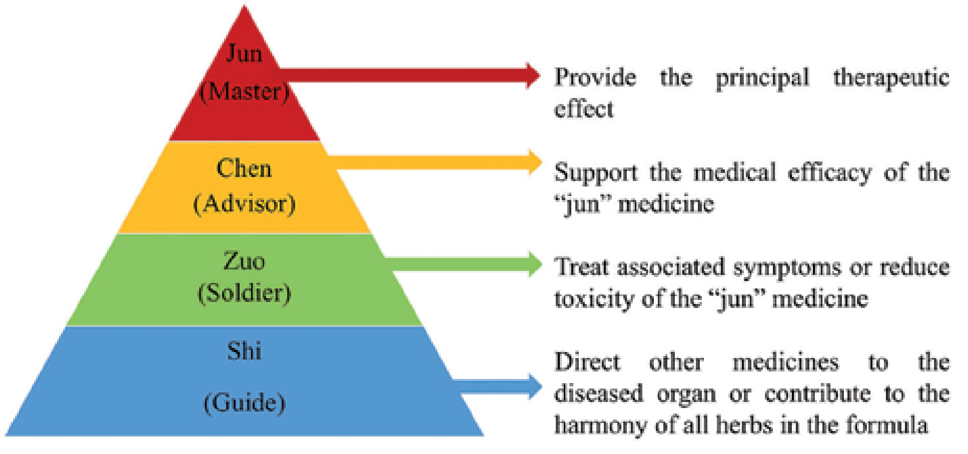

Figure 12.
The principle of 'Jun Chen Zuo Shi' theory [79].

instance, *Radix glycyrrhizae* (Gan cao 甘草) is the most commonly used 'shi' medicine, since its sweet flavour can improve the taste of formula decoction and enhance the harmony of the combined herbs [78]. For single-herb formulas, the medicinal herb is a 'jun' medicine (master medicine), which shows the principal medical effects.

For example, 'Ma-huang-tang' is a formula traditionally used to treat inflammatory liver disease and consists of *Ephedra sinica* Stapf (Ma huang 麻黄), *Ramulus cinnamomi Cassiae* (Gui zhi 桂枝), *Semen pruni Armeniacae* (Xing ren 杏仁) and *Radix glycyrrhizae* (Gan cao 甘草) (**Figure 13**). Ma huang works as the master medicine, which provides the main anti-inflammatory effects in the treatment of lung diseases. Gui zhi works as the advisor medicine and assists Ma huang to function well, which compiles the 'xiang shi' ('mutual enhancement') from the 'seven-relation compatibility'. Xing ren is the soldier medicine and enhances the efficacy of Ma huang. Gan cao functions as the guide medicine, which can adjust the properties of other medicines and improve the harmonisation in the formula. The overall efficacy of the TCM formula is a result of the mixture of chemical components in the multiple medicinal herbs [76]. However, it was reported that pure and active components which were separated and purified from single or multiple herbs are different in their chemical profile when compared to traditional decoctions [80]. This may be due to synergistic effects in the complex medicine or to chemical reactions and other changes which may occur during the decoction procedure.

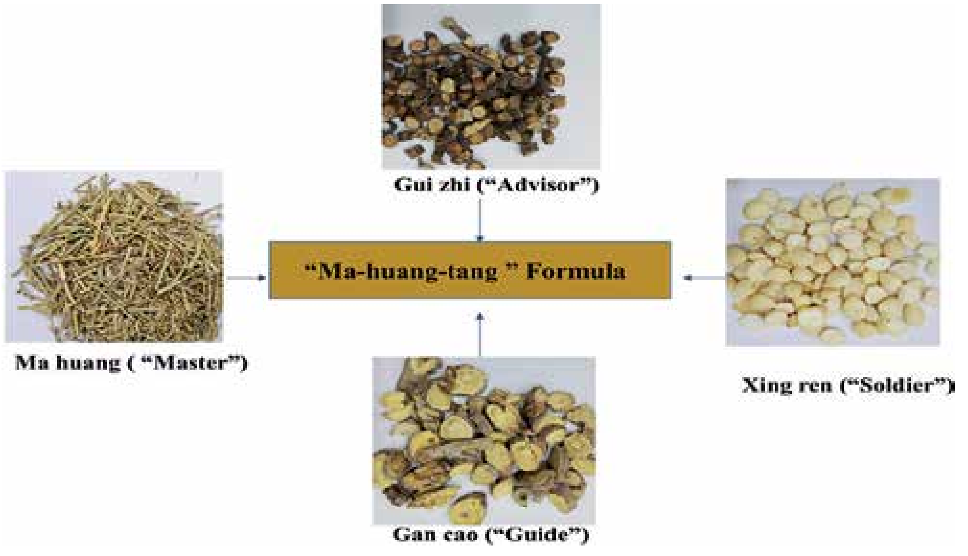

Figure 13.
Composition of herbs and roles in 'Ma-huang-tang' formula.

2.7 Formulations of TCM

2.7.1 Decoctions

Decoction is the earliest and most commonly used preparation method in the clinical application of TCM [81]. A TCM decoction is the herbal tea that is made of processed medicinal herbs which are simmered for hours [82]. The preparation of a traditional medicine decoction has multiple steps such as the sequence of boiling the herbal material and different boiling and filtering times. The preparation of a decoction is complex, as it requires experience and often takes considerable time. The herbal ingredients within the formula for decoction can be changed for the individual patient according to the disease. The herbal medicine may include leaves, flowers, roots, bark or fruits, and the procedure of preparation is depending on the part of the herb that is used. It is of concern that in some instances, the quality and stability of herbal material cannot be assured [14]. Also, the unpleasant flavour of a decoction has been a problem for patients for thousands of years. As equipment advances and market demand increases, modern decoctions have become very popular in TCM hospitals, because of convenience [83]. Decoctions have a quick and often complete absorption with good bioavailability [81]. In a modernised decoction method, the medicinal herbs are combined with water and are extracted in TCM decoction machines [83]. Modern formulations also include granules, oral liquids, tablets, capsules and injections. This change in methodology may in fact influence the composition of the final TCM, and this is another area that would benefit from comparative research studies directed at classical and modern decoction methods.

2.7.2 Modern granular formulations

The use of granules has dramatically increased in China since they are easy to handle, store and formulate. The granule made of a single herb is called a single dispensing granule; when made of multiple herbs, it is called a dispensing formula granule [84]. The preparation of granules is included in the extensive process of decoction: they are prepared by decoction or aqueous extraction with the aid of suitable excipients. After being dissolved in warm water, they can be ingested by patients. With the increasing development of TCM granules, international research has focused on whether decoction can be replaced by granules with similar effectiveness and safety [15]. During the procedure of granule preparation, the difference of water extraction, concentration, desiccation and granulation may influence the dissolution and chemical profiles of active ingredients [85]. Thus, this may result in different clinical efficacy between granules and decoction.

Recently, some cases related to the equivalence of effect between granules and decoction have been reported. Indeed, some differences in chemical consistency were identified, which suggest that the efficacy might not be equivalent between both forms of medicine. Ge-Gen-Qin-Lian-Tang is a classic TCM formula for the treatment of inflammatory bowel disease [86]. The comparison of chemical fingerprints of traditional decoction and granules of Ge-Gen-Qin-Lian-Tang was investigated by liquid chromatography-diode array detector (LC-DAD) to ensure the consistency of efficacy. The fingerprints demonstrated small variations among the 20 peaks, but the peak area of puerain, berberine and baicalein from the granule sample was 50% less than that of the decoction [87]. The TCM formula Da-Cheng-Qi-Tang is commonly used to treat digestive disease. The chemical consistency of decoctions of Da-Cheng-Qi-Tang prepared by traditional and modern methods was investigated. Five compounds were identified as chemical markers, an analysis of which established that the chemical fingerprints were not consistent between these two kinds of decoctions. The study showed that the traditional decoction method had a stronger purgative effect due to higher concentrations of rhein and sennosides [83]. Therefore, it is very important to understand the impact of the formulation method on the therapeutic effect of a final granular TCM product.

2.8 Fingerprint-efficacy analysis in TCM

Over the past 30 years, chromatographic and spectrophotometric fingerprinting methods have dramatically improved, and the application of hyphenated techniques such as high performance liquid chromatography (HPLC)-mass spectrometry (HPLC-MS), liquid chromatography-nuclear magnetic resonance (LC-NMR) or gas chromatography mass spectrometry (GC-MS) in the analysis of TCM facilitates the determination of the quality in research and in the pharmaceutical industry [4]. However, quality standards are challenging to establish, because the complexity of potential active ingredients in medicinal herbs is used in TCM. Today, commonly applied models for TCM quality control include conventional methods, such as microscopic, and macroscopic identification and comparison with monographs, chemical fingerprint analysis using thin-layer liquid chromatography (TLC), liquid chromatography (LC), HPLC, gas chromatography (GC), GC-MS, etc., and multiple marker assays.

Chemical fingerprints and bioactivities assay linked with multiple markers is currently recognised to be effective for TCM analysis [88]. The use of known and previously characterised markers for the analysis of constituents is the most popular method for identification and quality control in TCM. For example, the ginsenoside fingerprint profiles are applied for the authentication of Panax species [89]. Moreover, the fingerprint-efficacy relationship of Lycii fructus (Goji 枸杞) was studied by Zhang in 2018. The spectrum of ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) of three batches of Goji was correlated with the biological data using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. Compounds relating to the antioxidant activity of Goji, such as chlorogenic acid, quercetin and kaempferol, were subsequently identified by analysis of UPLC-MS/MS data [90].

Because of TCM's complexity, it is rare that any single ingredient provides the overall evidence for medical efficacy. Chemometric methods, such as similarity evaluation or principal component analysis, are often used to investigate the fingerprint and bioactivity relationship within the TCM [91]. GC-MS is usually applied for the analysis of volatile metabolites, whereas LC-MS is often used to identify and quantify non-volatile components. Hyphenated methods, such as LC-MS can provide information on fragments of constituents, which can help in the elucidation and identification of chemical structures. In recent years, LC-MS analysis has been widely used in TCM due to its high sensitivity, selectivity and generation of specific information [92]. NMR metabolomic profiling is currently recognised as a quick and generic method in the study and quality control of TCM material. For instance, it was reported that the quality control of *Radix Angelica Sinensis* was studied by NMR profiling [93], which resulted in observed differences between samples prepared by different methods.

To ensure the consistency of herbal medicine, the concept of phyto-equivalence was developed. Phyto-equivalence is a comparison of the chromatographic fingerprint of a herbal medicine with the profile of a standard reference product. The fingerprint is defined as a characteristic profile, which reflects the overall complex chemical composition of the sample analysed by chromatographic or electrophoretic techniques. Chromatographic fingerprints are accepted by the US Food and Drug Administration (FDA) in applications for new product approvals [94]. In TCM studies, fingerprints could provide a complete set of information of chemical components including the relative quantity of all detectable analytes [95]. Fingerprinting is widely used to authenticate or differentiate between species, geographical regions or processing methods in TCM.

The 'fingerprint-efficacy relationship' is a method that associates TCM fingerprints with a specific pharmacological effect [96]. Multiple methods relate chemical fingerprints, such as characteristic peaks, to bioactivities. The 'fingerprint-efficacy relationship' investigation procedure (**Figure 14**) involves finding the appropriate analytical methods for fingerprints, identifying assays for the various bioactivities, using statistical methods to find the fingerprint-efficacy relationship, select candidate components, and validation of the bioactivities of the identified candidate

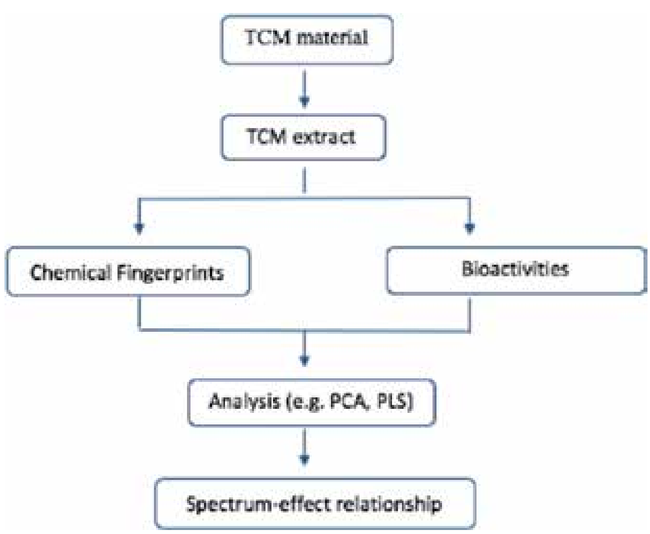

Figure 14.
The method of 'fingerprint-efficacy relationship' in TCM [88].

components [88]. For instance, *Valeriana jatamansi* Jones (Zhi zhu xiangI 蜘蛛香) has a long history of use in TCM to treat mood disorders like anxiety. The 'fingerprint-efficacy relationship' was studied by correlating HPLC fingerprints with *in vitro* and *in vivo* tests. Four chemical components, hesperidin, isochlorogenic acid A, isochlorogenic B and isochlorogenic C are regarded as multiple biological markers for this anti-anxiety effect [97].

Principal component analysis (PCA) is a statistical method, which can reduce the number of variables and dimensionality and create principal components (PCs) to explain the variables in original data. PCA is usually combined with cluster analysis, correlation analysis or regression analysis to determine the relationship of fingerprints and efficacy. It extracts data and can remove redundant information to focus on the main factors. PCA is often used together with other chemometric methods due to the lack of a specific quantification mathematical model for variables [98]. For example, *Andrographis herba* (Chuan xin lian 穿心莲) is well-known in China because of its bitter taste, which is one of the five flavours in TCM theory. The bitter flavour is recognised as relating to the pharmacological effects of *Andrographis herba*. In Zhang's group, the chemical components of 30 different types of *Andrographis herba* and fingerprint spectrum were determined by HPLC, and the PCA was applied to analyse the chemical components relating to bitter taste relating. According to the results from PCA, andrographolide, neoandrographolide, 1,4-deoxyandrographolide and dehyandrographlide were determined as substances responsible for the bitter flavour of Chuan xin lian [99]. *Ephedra sinica* Stapf (Ma huang 麻黄) is commonly applied for rheumatism, asthma, fever and rheumatoid arthritis in China. The significance of the inorganic elements of Ma huang from different geographical regions was studied by using elemental plasma mass spectroscopy fingerprints and PCA, and the study showed that this is an effective strategy to discriminate TCM samples [100].

3. Conclusion

The therapeutic formulae used for health maintenance and disease treatment in TCM are often complex mixtures whose chemical fingerprints are influenced by many factors, by the ancient practices of Daodi cultivation, Paozhi processing and Fangji combinations. As TCM undergoes modernisation and seeks increased entry to international markets which present rigorous legislative barriers, the quality, safety and efficacy are under increasing scrutiny.

In addition, there are increasing demands on supply, and attention needs to be focused on sustainable production, including mass cultivation and appropriately structured wild harvesting. It is obvious from ongoing research that the ancient processes, which vary across the land mass of China, influence the chemical fingerprint of the resulting therapeutic formulae, and thus render TCMs with the same name, from different regions, to have different potency.

Going forward, it is important for the scientific community to continue to apply sophisticated methodologies and chemometric analyses toward understanding how the ancient TCM processes impact on the final product and how sustainable practices can be implemented that will lead to standardised therapeutic formulae which reach the international standards of quality, safety and efficacy.

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Author details

Jinfan Wang, Astrid Sasse and Helen Sheridan*
School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

*Address all correspondence to: hsheridn@tcd.ie

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Chapter 3

Antioxidant Activity of Faba Bean Extracts

Wojciech Rybiński, Magdalena Karamać, Katarzyna Sulewska and Ryszard Amarowicz

Abstract

Phenolic compounds were extracted from seeds of 22 cultivars of faba bean (*Vicia faba* L.) by using 80% (v/v) aqueous acetone. The total phenolic compound and condensed tannins contents of the extracts and their antioxidant activity were determined using the Folin-Ciocalteu's phenol reagent, vanillin/HCl method, and ABTS and FRAP assays, respectively. The content of total phenolic compounds ranged from 40.7 to 66.1 mg/g extract and from 326 to 574 mg/100 g seeds. Contents of condensed tannins ranged from 2.40 to 49.9 mg/g extract and from 22.2 (FAB) to 365 mg/100 seeds. The extracts and seeds were characterized by Trolox equivalent antioxidant capacity (TEAC) values ranging from 0.550 (FAB 443) to 1.030 mmol Trolox/g extract (FAB 187) and from 4.85 (FAB 318) to 9.81 mmol Trolox/100 g seeds (FAB 187). Ferric-reducing antioxidant power (FRAP) values varied from 0.595 (FAB 443) to 0.908 mmol Fe^{2+}/g extract (FAB 5023) and from 4.61 (FAB 297) to 7.90 mmol Fe^{2+}/100 g seeds (FAB 187). The total phenolic content of faba bean extract was correlated with the results of the ABTS (r = 0.864) and FRAP (r = 0.862) assays. The coefficients of correlations between the contents of condensed tannins and ABTS and FRAP results were 0.543 and 0.862. We also noted a correlation between results of ABTS and FRAP assays (r = 0.795).

Keywords: faba bean, *Vicia faba* L., phenolic compounds, antioxidant activity, ABTS assay, DPPH assay

1. Introduction

Phenolic compounds of plant origin can inhibit or delay the oxidation of nutrients present in food products. In the human organism, natural antioxidants can protect lipids, proteins, and DNA against reactive oxygen and nitrogen species (ROS, RNS) [1, 2]. Results of numerous research groups demonstrate the protective effect of consuming phenolic-rich grains, legumes, oilseeds, fruits, berries, and nuts against several chronic diseases [3–7].

In human nutrition, legumes are an important source of proteins, starch, oligosaccharides (prebiotics), dietary fibers, vitamins, and minerals [8, 9]. As a rich source of natural antioxidants, legumes can play an important role in cardio and cancer protection [5, 10–15].

Faba bean (*Vicia faba*) is a species of Fabaceae family. It is native to South America, North Africa, and southwest and south Asia and is extensively cultivated elsewhere. Similar to other legumes, faba bean seeds contain phenolic compounds

[16] including condensed tannins [17–19]. The antioxidant potential of faba bean was determined using DPPH, FRAP, and ORAC assays [17, 20, 21]. Very high antioxidant capacity of *Vicia faba* sprouts was confirmed by Okumura et al. [22].

2. Experimental

2.1 Material

Plant material consisted of a collection of 22 faba bean accessions derived from Syria, Morocco, Tunisia, Sudan, Egypt, Yemen, Israel, Georgia, Azerbaijan, Tajikistan, Mongolia, Afghanistan, India, Australia, and Poland (**Table 1**). Information about seed coat color and 100 seed weight was provided on the basis of results obtained in a field experiment conducted in Cerekwica (Poland, 51°55′N, 17°21′E).

Description of faba bean seeds is reported in **Table 1**.

2.2 Chemicals

Ferrous chloride, sodium persulfate, ferrous chloride, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS),

No.	Accession code	Country of origin	Seeds coat color	Weight of 100 seeds (g)
1	FAB 337	Syria	Dark	47.2
2	FAB 443	Syria	Bright	76.6
3	FAB 5023	Morocco	Bright	45.8
4	FAB 5019	Morocco	Bright	52.1
5	FAB 6440	Tunisia	Bright	68.0
6	FAB 6441	Tunisia	Bright	65.0
7	FAB 225	Sudan	Bright	64.7
8	FAB 297	Sudan	Bright	45.0
9	FAB 6474	Egypt	Bright	27.4
10	FAB 219	Yemen	Bright	57.0
11	FAB 6318	Israel	Bright	81.5
12	FAB 344	Israel	Dark	46.8
13	FAB 604	Georgia	Bright	66.3
14	FAB 294	Azerbaijan	Bright	43.4
15	FAB 354	Tajikistan	Bright	40.0
16	FAB 202	Mongolia	Bright	68.4
17	FAB 144	Afghanistan	Dark	65.5
18	FAB 187	Afghanistan	Bright	30.9
19	FAB 250	India	Bright	38.6
20	FAB 446	India	Bright	41.1
21	FAB 7077	Australia	Bright	72.5
22	Martin	Poland	Bright	55.8

Table 1.
Description of faba bean seeds.

6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), Folin–Ciocalteu's phenol reagent, and (+)-catechin were purchased from Sigma (Poznań, Poland). Methanol, acetone, and hexane were obtained from P.O.Ch. Company (Gliwice, Poland).

2.3 Extraction

Phenolic compounds were extracted from grounded and defatted with hexane faba bean seed by using 80% acetone (v/v) at a solid to solvent ratio of 1:10 (w/v), for 15 min at 50°C [23]. Extraction was carried out in flasks placed in a shaking water bath (Elpan 357, Wrocław, Poland). Acetone from the combined extract was evaporated using a Büchi rotary evaporator. The sample was then freeze-dried.

2.4 Determination of total phenolic compound contents

The content of total phenolic compounds in the extracts was determined using the Folin–Ciocalteu's phenol reagent [24]. The results were expressed as (+)-catechin equivalents per g of the extract and per 100 g of seeds.

2.5 Determination of condensed tannins content

The content of condensed tannins was determined using a vanillin/HCl colorimetric method [25]. The results obtained were reported as mg catechin equivalent per g extract and 100 g of seeds.

2.6 ABTS assay

The Trolox equivalent antioxidant capacity (TEAC) was determined using the method of Re et al. [26]. The results obtained were reported as mmol Trolox equivalents per g extract and 100 g of seeds.

2.7 FRAP assay

The ferric-reducing antioxidant power (FRAP) assay was performed as previously described by Benzie and Strain [27]. The results obtained were reported as mmol Fe^{2+} equivalents per g extract and 100 g of seeds.

2.8 Statistical analysis

The results are reported as a mean value of three determinations ± standard deviation. The Pearson correlation was used to determine the relation between results of total phenolics, condensed tannins, TEAC, and FRAP values. Moreover the principal component analysis (PCA) and hierarchical cluster analysis (HCA) with Ward's method using Euclidean distances were conducted. Statistical and chemometric analyses of data were performed using the Statistica software (Windows software package 8.0).

3. Results and discussion

3.1 Content of total phenolics and condensed tannins

The content of total phenolics in the extracts was determined using the Folin–Ciocalteu's phenol reagent. The results were expressed as (+)-catechin equivalents per g of the extract or 100 g seeds. The results are reported in **Tables 2** and **3**. The content of total phenolics ranged from 40.7 (FAB 219) to 66.1 mg/g extract (FAB 187) and from 326 (FAB 219) to 574 mg/100 g seeds (FAB 5019).

The content of condensed tannins in the extracts was determined using the vanillin/HCl colorimetric method. The results ranged from 2.40 (FAB 297) to 49.9 mg/g extract (FAB 225) and from 22.2 (FAB 297) to 441 mg/100 seeds (FAB 5019) (**Tables 1** and **3**).

The results obtained in this research confirm the fact that faba bean is a rich source of phenolic compounds as well as condensed tannins. A lower content of total phenolic compounds was previously reported for extracts of pea [28], white bean [29], broad bean [23, 30] lupin [31], and grass pea [32]. A similarly high content of total phenolic compounds was previously reported for red lentil [33], green lentil [34], red bean [35], and adzuki bean [36].

The presence of condensed tannins in faba bean seeds determined with the vanillin method was reported by Amarowicz et al. [37], Baginsky et al. [38], Amarowicz and Shahidi [39], and Zduńczyk et al. [17]. In a research conducted by

No	Total phenolics[a] (mg/g extract)	Condensed tannins[b] (mg/g extract)	TEAC (mmol TE/g extract)	FRAP (mmol Fe^{2+}/g extract)
1	51.5 ± 0.4	26.4 ± 0.7	0.656 ± 0.014	0.673 ± 0.014
2	42.1 ± 1.0	10.9 ± 0.9	0.550 ± 0.016	0.535 ± 0.010
3	61.2 ± 0.5	25.9 ± 1.4	0.860 ± 0.011	0.908 ± 0.012
4	62.8 ± 2.4	48.2 ± 1.3	0.854 ± 0.022	0.753 ± 0.008
5	60.5 ± 1.6	43.2 ± 1.8	0.775 ± 0.023	0.742 ± 0.011
6	47.6 ± 0.2	21.5 ± 1.1	0.563 ± 0.008	0.634 ± 014
7	60.2 ± 0.7	49.9 ± 0.7	0.886 ± 0.017	0.715 ± 0.015
8	46.9 ± 0.9	2.40 ± 0.72	0.710 ± 0.018	0.572 ± 0.013
9	41.8 ± 0.4	8.77 ± 1.06	0.669 ± 0.014	0.636 ± 0.009
10	40.7 ± 1.4	4.32 ± 0.78	0.661 ± 0.012	0.603 ± 0.016
11	45.5 ± 0.3	4.15 ± 0.69	0.592 ± 0.011	0.651 ± 0.014
12	55.0 ± 1.6	22.1 ± 1.6	0.826 ± 0.022	0.734 ± 0.012
13	59.1 ± 1.1	21.3 ± 1.5	0.806 ± 0.020	0.807 ± 0.011
14	46.8 ± 1.0	35.3 ± 2.1	0.691 ± 0.004	0.671 ± 0.011
15	61.1 ± 1.4	49.1 ± 1.4	0.844 ± 0.017	0.824 ± 0.014
16	51.7 ± 1.5	28.2 ± 1.3	0.841 ± 0.024	0.703 ± 0.012
17	46.3 ± 1.5	23.8 ± 1.4	0.721 ± 0.014	0.588 ± 0.008
18	66.1 ± 1.9	32.5 ± 1.3	1.035 ± 0.014	0.833 ± 0.009
19	47.9 ± 1.0	14.4 ± 0.8	0.631 ± 0.011	0.718 ± 0.013
20	48.8 ± 1.7	47.8 ± 2.3	0.694 ± 0.009	0.675 ± 0.010
21	48.7 ± 0.6	34.2 ± 1.5	0.631 ± 0.010	0.694 ± 0.015
22	65.7 ± 1.9	39.9 ± 1.1	0.894 ± 0.015	0.807 ± 0.007

[a]*Catechin equivalents.*
[b]*Catechin equivalents.*

Table 2.
Contents of total phenolic compounds and condensed tannins and antioxidant activity of faba bean extracts. Results are reported per g of extract.

No	Total phenolics[a] (mg/100 g seeds)	Condensed tannins[b] (mg/100 g seeds)	TEAC (mmol TE/100 g seeds)	FRAP (mmol Fe^{2+}/100 g seeds)
1	484 ± 4	247 ± 6	6.16 ± 0.13	6.32 ± 0.13
2	421 ± 10	109 ± 19	5.50 ± 0.16	5.35 ± 0.10
3	524 ± 5	222 ± 12	7.37 ± 0.09	7.78 ± 0.10
4	574 ± 22	441 ± 12	7.80 ± 0.20	6.88 ± 0.08
5	509 ± 13	362 ± 15	6.51 ± 0.20	6.23 ± 0.09
6	425 ± 2	192 ± 9	5.82 ± 0.07	5.66 ± 0.13
7	539 ± 6	447 ± 6	7.94 ± 0.15	6.40 ± 0.13
8	378 ± 7	22.2 ± 1.0	5.72 ± 0.15	4.61 ± 0.10
9	395 ± 4	82.8 ± 9.9	6.31 ± 0.13	6.01 ± 0.08
10	326 ± 11	34.5 ± 7.5	5.28 ± 0.10	4.82 ± 0.13
11	373 ± 2	34.0 ± 4.9	4.85 ± 0.09	5.34 ± 0.11
12	475 ± 13	191 ± 14	7.13 ± 0.19	6.34 ± 0.10
13	559 ± 9	201 ± 14	7.63 ± 0.19	7.64 ± 0.10
14	410 ± 9	309 ± 18	6.04 ± 0.03	5.85 ± 0.12
15	561 ± 13	451 ± 13	7.75 ± 0.16	7.57 ± 0.11
16	486 ± 14	265 ± 12	7.91 ± 0.22	6.61 ± 0.08
17	421 ± 14	217 ± 12	6.56 ± 0.13	5.34 ± 0.08
18	626 ± 18	308 ± 13	9.81 ± 0.14	7.90 ± 0.12
19	418 ± 9	126 ± 7	5.52 ± 0.09	6.28 ± 0.09
20	482 ± 17	471 ± 23	6.85 ± 0.09	6.66 ± 0.15
21	464 ± 6	326 ± 14	6.01 ± 0.10	6.60 ± 0.07
22	602 ± 17	365 ± 10	8.20 ± 0.14	7.40 ± 0.10

[a]*Catechin equivalents.*
[b]*Catechin equivalents.*

Table 3.
Contents of total phenolic compounds and condensed tannins and antioxidant activity of faba bean seeds. Results are reported per 100 g of seeds.

Luo et al. [40], the content of condensed tannins in faba bean extracts ranged from 0.9 to 1.9 g of gallic acid equivalents/100 g extract. In this research, the authors used the Folin-Ciocalteu's phenol reagent to determine the content of tannins precipitated from the extract by using polypyrrolidone (PVPP). Amarowicz and Shahidi [39] identified gallate procyanidin dimer and three gallate procyanidins in faba bean extract by using HPLC-DAD-MS. The content of the abovementioned compounds was 689, 89.8, 28.6, and 18.3 μg/g extract. Five procyanidin dimmers and three procyanidin trimers were determined in faba bean extract using an UHPLC-ESI-QTOF-MS method [41]. The presence of procyanidin B1, B2, B3, B4, C1, and C2 has been reported by De Pascual-Teresa et al. [42].

3.2 Antioxidant activity

Antioxidant properties of the extracts were investigated using ABTS and FRAP assays. The extracts and seeds were characterized by the Trolox equivalent antioxidant capacity (TEAC) values ranging from 0.550 (FAB 443) to 1.030 mmol Trolox/g extract (FAB 187) and from 4.85 (FAB 318) to 9.81 mmol Trolox/100 g seeds (FAB 187). Ferric-reducing antioxidant power (FRAP) values varied from 0.595 (FAB 443) to 0.908 mmol Fe^{2+}/g extract (FAB 5023) and from 4.61 (FAB 297) to 7.90 mmol Fe^{2+}/100 g seeds (FAB 187).

The results of ABTS assay obtained in this study for faba bean extracts were much higher than those reported before for extracts of grass pea (0.017–0.037 mmol Trolox/g) [32], cow pea (0.285–0.665 mmol Trolox/g) [43], white bean (0.0270–0.043 mmol Trolox/g) [29], mung bean (0.021–0.031 mmol Trolox/g) [35], and lupin (0.260–0.620 mmol Trolox/g) [31]. The results of FRAP assay were also much higher than those reported for extracts of grass pea (0.045–0.120 mmol Fe^{2+}/g), [32], cow pea (0.487–1.566 mmol Fe^{2+}/g) [43], white bean (0.066–0.089 mmol Fe^{2+}/g) [29], and lupin 0.046–0.064 [31].

3.3 Statistical analysis

In our study, for the first time, a correlation was calculated between the content of phenolic compounds in the faba bean extracts and their antioxidant activity. The

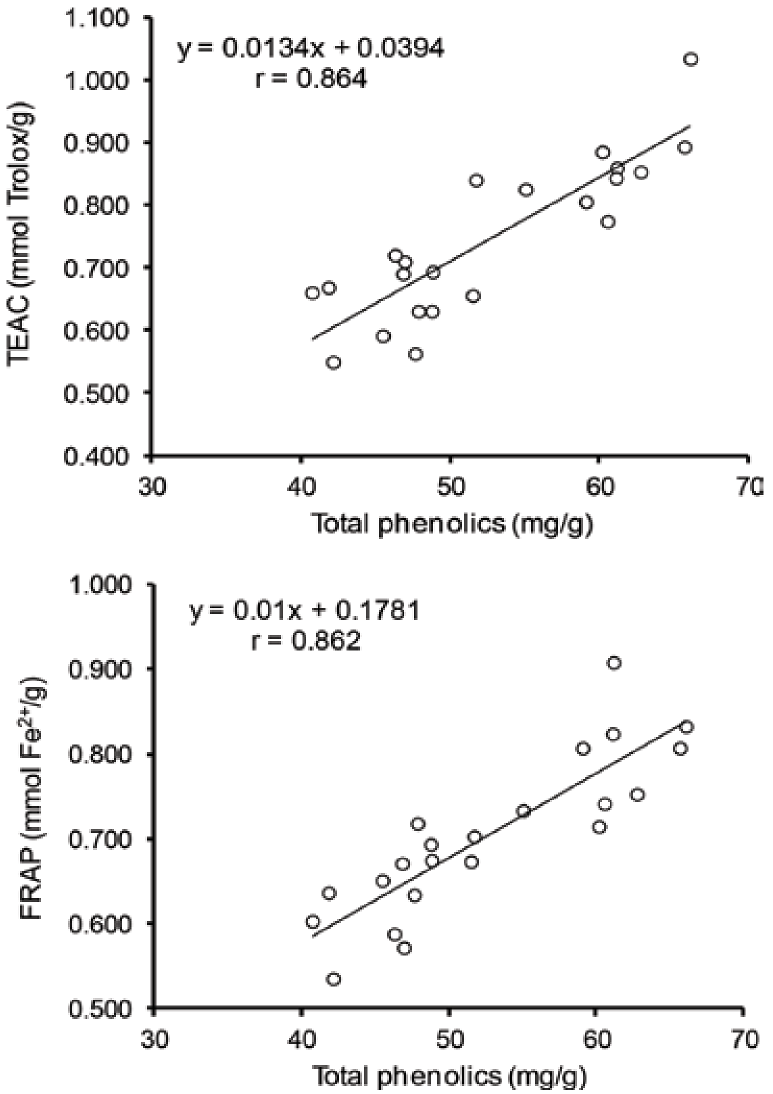

Figure 1.
Correlation between the total phenolic contents and the results of ABTS and FRAP assays.

coefficients of correlation between the total phenolic content and the results of the ABTS and FRAP assays were 0.864 and 0.862, respectively (**Figure 1**). The correlations between the content of condensed tannins and results of ABTS and FRAP assays were weaker and characterized by r = 0.543 and r = 0.528 (**Figure 2**). A correlation was also observed between the results of both assays (r = 0.795) (**Figure 3**).

In our previous study, we noted a correlation between the total phenolic content and ABTS and FRAP results determined for red bean (r = 0.997 and 0.997, respectively) [35], white (r = 0.480 and 0.850, respectively) [29], and grass pea (r = 0.881 and 0.781, respectively) [32]. Statistically significant correlations between the content of total phenolics and TEAC as well as between the content of condensed tannins and TEAC (r = 0.857 and 0.787, respectively) were reported for extracts obtained from seeds of faba bean, broad bean, adzuki bean, red bean, pea, red lentil, and green lentil [44].

In the principal component analysis (PCA) (**Figure 4**), the two first components accounted for 93.6% of the total variability between the faba bean cultivars. The analysis includes the content of total phenolic compounds and condensed tannins

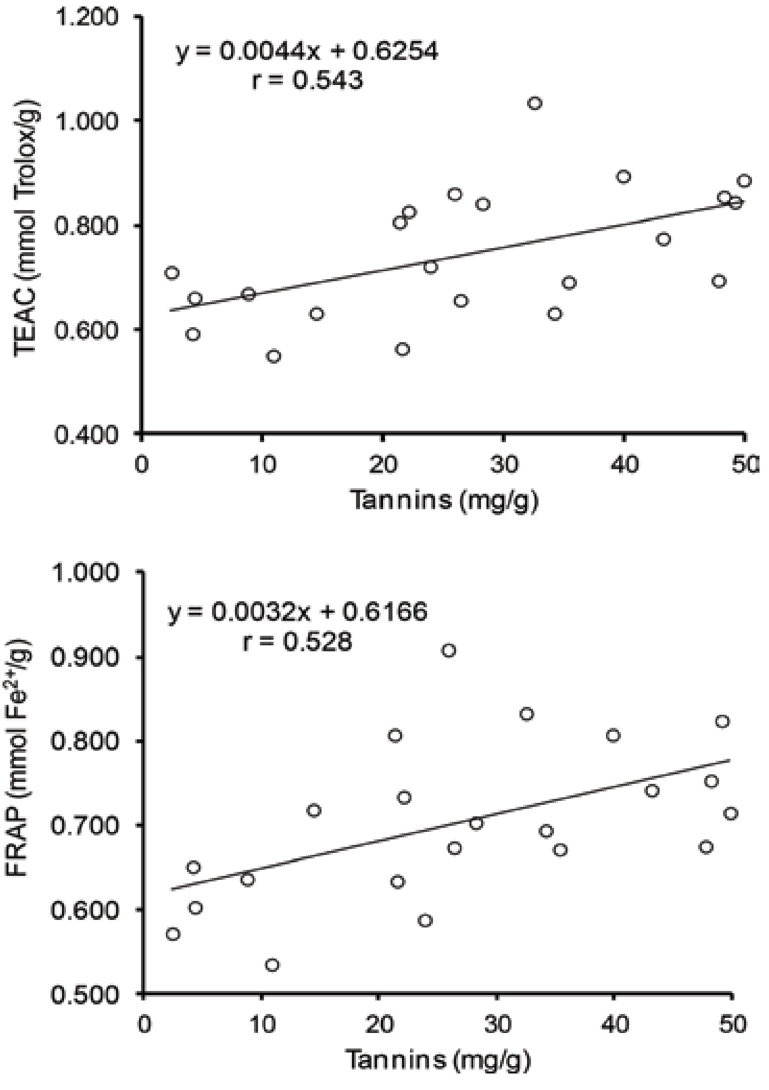

Figure 2.
Correlation between the content of tannins and the results of ABTS and FRAP assays.

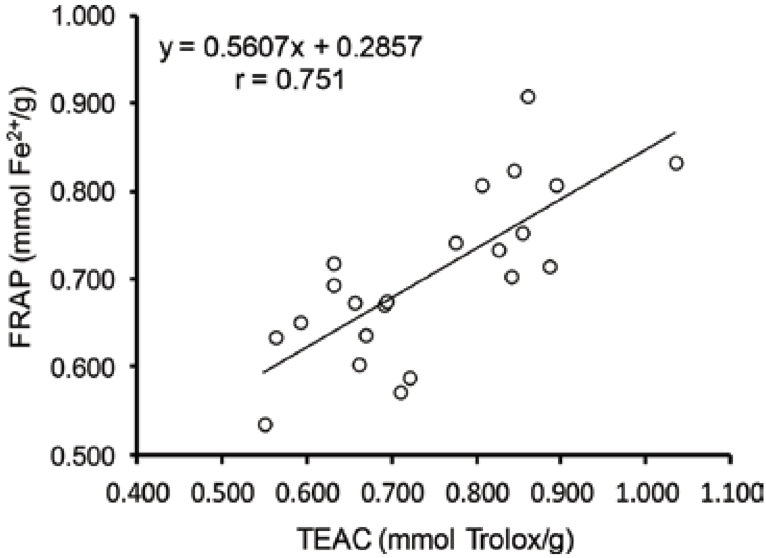

Figure 3.
Correlation between the results of FRAP and ABTS assays.

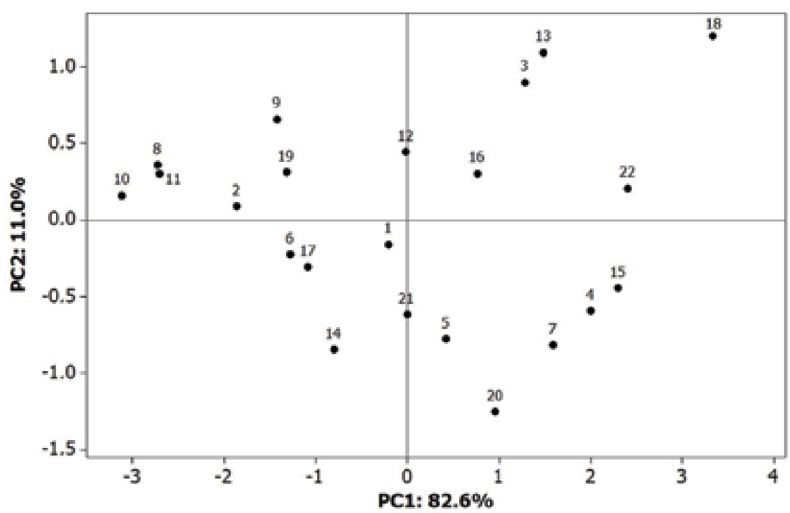

Figure 4.
Results of the principal component analysis (PCA).

in seeds as well as their antioxidant potential determined using ABTS and FRAP methods. A considerable variability in terms of the analyzed traits expressed jointly with the grates Mahalanobis distance was recorded for sample 18 from Afghanistan (FAB 187) and samples 8, 10, and 11 from Sudan (FAB 297), Yemen (FAB 219), and Israel (FAB 6318). The close clustering was observed for seeds from Sudan, Yemen, and Afghanistan and for seeds from Morocco, Sudan, and Tajikistan (sample 4, 7, and 15; FAB 5019, FAB 225, and FAB 354).

The hierarchical cluster analysis (**Figure 5**) showed several pairs of faba bean accessions (e.g., FAB 337 and FAB 202, FAB 5023 and FAB 604). Two main clusters were observed. The first contained 16 accessions, whereas the second contained only 6. The presence of similar pairs of faba bean accessions from different countries confirms the limitation of the hierarchical cluster analysis in the discrimination of the geographical origin of samples.

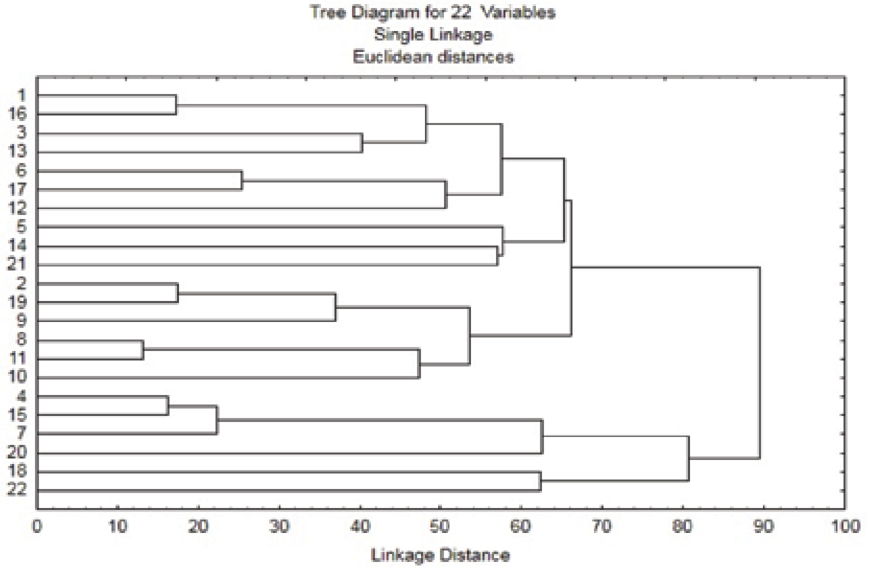

Figure 5.
The hierarchical cluster analysis.

4. Conclusions

The extracts obtained from faba bean were characterized by a high content of phenolic compounds and condensed tannins. Their antioxidant potential was higher than that reported previously for the extracts of other legume seeds. The content of total phenolics and condensed tannins in the faba bean extracts strongly influenced the antioxidant activity of extracts determined using ABTS and FRAP assays.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Wojciech Rybiński[1], Magdalena Karamać[2], Katarzyna Sulewska[2] and
Ryszard Amarowicz[2]*

1 Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

2 Institute of Animal Reproduction and Food Research,
Polish Academy of Sciences, Olsztyn, Poland

*Address all correspondence to: r.amarowicz@pan.olsztyn.pl

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Chapter 4

Antiviral Plant Extracts

Ganesh Kumar Anbazhagan, Sankarganesh Palaniyandi and Baby Joseph

Abstract

Herbal therapy has been an important part of health and wellness for hundreds of years. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world. Recent surveys have indicated that worldwide people now make more visits to healthcare professionals who specialize in alternative medicine than to doctors who practice conventional medicine. It is hoped that extracts from herbs will add new medicinal plants to the world's pharmacopoeia before they are lost forever that too particularly against viral infection. Based on the ethnobotanical data and the information collected from Siddha/Ayurvedic practitioners of India, extracts obtained from *Boerhavia diffusa*, *Eclipta alba* and *Phyllanthus amarus* will be having great potential in curing various viral infections.

Keywords: antiviral, herbs, *Boerhavia diffusa*, *Eclipta alba*, *Phyllanthus amarus*

1. Introduction

For hundreds of years, herbal therapy has played a major role in maintaining the health and wealth of people around the world. For treatment of various illnesses, herbs are widely used. In most of the developing countries, nowadays, people prefer alternative medicine than conventional medicine indicated by a recent survey. Many publications and books claim the proficiency of herbs for the treatment of various illnesses including cirrhosis, hepatitis, and other deadly diseases. It has been observed widely that the medicinal plants and traditional medicines are used as a normative basis for maintaining good health [1].

In the process of developing many drugs, an increasing demand for the use of medicinal plant-derived products in the industries has been found [2]. For personal health maintenance, herbal remedies have become more popular in treatment of minor ailments. Due to increasing demand, there is a great risk that the existing medicinal plants on earth might disappear. Thus an important component of the health care system in a country like India is medicinal plants.

Chemical compounds obtained from plant source are termed as secondary plant products. Alkaloids and glycosides are the two major compounds present. In 4000 plant species, more than 3000 alkaloids have been identified. The difference between a toxic and medicinal effect of many alkaloids depends upon the dosage. When a sugar molecule gets attached to component which is active, it is called as glycoside which is categorized by the nature of the active component or nonsugar.

Clinical research of the present era has confirmed the ability of many medicinal plants in treating various viral diseases, while many recent scientific research have discovered the exact mechanism by which many different plants provide their therapeutic advantages. Three such plants are *Boerhavia diffusa*, *Eclipta alba* and *Phyllanthus amarus*.

2. Boerhavia diffusa

Boerhavia diffusa is a herbaceous plant commonly known as punarnava in Sanskrit. The whole plant is known to have medicinal properties and being used in most parts of the subcontinent. Alkaloids are best known principle product present in the roots which is known as punarnavine. In traditional medicine, *Boerhavia diffusa* is more commonly used in the treatment of jaundice, dyspepsia, abdominal pain, spleen enlargement and as an antistress agent. In the year 1980–1991, researchers proved that *B. diffusa* root extract had strong antihepatotoxic properties which can be used to cure viral hepatitis [3].

2.1 Origin and distribution

B. diffusa is found mostly in Asia, that too in warmer parts with an altitude of 2000. It grows well in crop fields and wastelands after the rainy season [4]. The genus *Boerhavia* has many species, and is also found spread in few regions of the world. *B. diffusa* is found in few places of Australia, South Africa and USA apart from Asian countries like China, Egypt, Pakistan, Sudan, Sri Lanka and in several countries of the Middle East [5]. Out of the 40 species of this genus, 6 species are found in India namely *B. diffusa*, *B. chinensis*, *B. erecta*, *B. repens*, *B. rependa* and *B. rubicunda* [6].

Taxonomy

Kingdom	-	Plant Kingdom
Class	-	Dicotyledons
Sub class	-	Monochlamydeae
Series	-	Curvembryae
Order	-	Caryophyllales
Family	-	Nyctaginaceae
Genus	-	*Boerhavia*
Species	-	*diffusa*
Binomial name	-	*Boerhavia diffusa*

Floral formula

Br, Brl, O, [+], P5, A[5], G[2]

2.2 Chemical constituents

The main component 2-glucopyranose-4-hydroxy-5-[P-hydroxyphenyl]-propionyldiphenyl methane was found in the roots of the plant. Many steroids, triterpenoids, alkaloids, flavonoids, lipids, lignins, proteins, carbohydrates and glycoproteins are mostly found [7]. Punarnavine, hypoxanthine 9-L-arabinofuranoside, ursolic acid, boeravinone, punarnavoside and liirodendrin have been purified and elucidated in detail for their biological activity with antiviral properties. Large amount of potassium nitrate is mostly found in this plant. The entire plant has huge percentage of proteins and fats. It also contains 14 amino acids in root, out of which 7 are essential amino acids. Punarnavoside is an antifibrinolytic compound isolated from the roots of *B. diffusa* long back [8]. When roots were analyzed for phytochemical screening, maximum alkaloid content [2%] was found.

2.3 Pharmacology

Pharmacological studies have demonstrated strong antiviral properties. Wide diuretic properties have been found in punarnavoside obtained from Punarnava, apart from that anthelmintic, antifibrinolytic, anticonvulsant, antibacterial, antihepatotoxic, antiasthmatic and antinematodal activity have also been observed. Against various abnormal production of liver enzymes AST, ALT and pigment bilirubin, the aqueous root extracts of *B. diffusa* at a dose of 2 ml/kg exhibited strong protection [9].

Drugs obtained from this herb, given either alone or in combination with other drugs were found to be effective in killing many virus for disorders pertaining to liver, infections of the respiratory tract and heart disease [10]. Against various bacteria, the purified form of glycoprotein obtained from *B. diffusa* exhibited strong antimicrobial activity. Many clinical researches have proved that against bacteriophages punarnava is found to be very effective [11]. For inducing systemic resistance in various crops against different viruses, the roots of *B. diffusa* are used [12].

2.4 Antiviral activity of *Boerhavia diffusa*

Against potato virus X, *Boerhavia diffusa* have shown a very good effect in inhibiting in hypersensitive and systemic hosts. The inhibition of these extracts was found to be very sensitive to actinomycin D. By the application of the aqueous root extract of *B. diffusa*, the destructive yellow mosaic disease of mung bean [*Vigna radiata*], caused by mung bean yellow mosaic virus was controlled. The treatment also resulted in increased plant height, root nodulation, primary and secondary branches, grain yield and pod formation. Against bacteriophage, the purified form of glycoprotein obtained from *Boerhavia diffusa* showed strong antimicrobial activity. To resist viral infection in many susceptible crops, the extracts obtained from the roots of *B. diffusa* was used. This antiviral agent was active against *Nicotiana tabacum*, Sunhemp and *Crotalaria juncea* [13].

3. *Eclipta alba*

Eclipta alba grows as a weed commonly in wet places around the world. It is widely distributed in India, China, Thailand and Brazil. *Eclipta alba* [L.] Hassk. [syn. *Eclipta prostrata* L.], commonly known as False Daisy is a plant from the family Asteraceae. Different varieties of indigenous drugs possessing analgesic properties have been obtained from this plant in Ayurveda. Extract of *E. alba* showed strong analgesic properties [14].

3.1 Origin and distribution

In the tropical and subtropical regions of the earth. *Eclipta alba* is usually found on poorly drained wet areas, on the dikes of rice paddies, along streams and ditches in marshes. It is most commonly found in elevated lands with 1200 mm of rainfall or more. It grows very well in salty and humid conditions but is mostly a plantation crop [15]. In few countries, it is found near sea level and is said to be a naturalized weed in waste places, open fields often near ditches and in cultivated areas [16]. In some parts of the world, it is a common plant of foreshores, streamsides, wet situations, weed of wet pasture and in roadside ditches. In is also found in low altitudes [17].

Taxonomy
Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Asterales
Family - Asteraceae
Genus - *Eclipta*
Specie - *alba*
Binomial name - *Eclipta alba*
Floral formula
Br, Brl, O, %, Kα, C[5], A[5], G[2]

3.2 Chemical constituents

In the year 1991, a new aldehyde was isolated from this plant. The leaves of this plant contain a-terthienyl methanol, 2-formyl-terthienyl, stigmasterol, de-me-wedelolactone 7-O-glucosides and wedelolactone [18]. Aerial parts of the plant contain β-amyrin, wedelolactone, phytosterol A, luteolin-7-glucoside and its glucosides [19].

3.3 Pharmacology

In Ayurveda and in traditional Chinese herbal medicine, this species is widely used. It is used as liver tonic, rejuvenative and also widely used for better growth of hair [20]. The whole plant is used as ophthalmic, purgative, astringent, deobstruent, depurative, emetic, febrifuge, tonic and styptic. It is used internally for the treatment of anemia and diphtheria, dropsy and liver complaints [21], tinnitus, premature greying of the hair and tooth loss. It is also widely used for many external applications like oil for hair loss, dermatitis, wounds and even for athlete's foot [22]. For treating scorpion stings, its leaves are used. The plant juice is widely used for catarrhal problems and for those having increased production of bilirubin.

At the time of flowering, this plant is mostly harvested and is dried for further use. The roots are purgative and emetic. In cattle, it act as antiseptic to cure ulcers and wounds. In Ayurvedic medicine, the leaves of this plant are used a liver tonic. The dye produced from *E. alba* is most widely used for the purpose of tattooing [23].

To treat eczema and dermatitis, *E. alba* is widely used. In China and Brazil, it is also used as antivenom against snakebite. To reduce the inflammation of spleen and liver on few occasions, *E. alba* was used and found to be effective. Antiviral activity particularly in inhibiting Ranikhet disease virus, the alcoholic extract of the plant was proved to be very effective. To promote bile flow and protect the parenchyma, it is commonly used in viral hepatitis. This plant was widely used for antiaging too. In case of debility, the tonic obtained particularly from *Eclipta alba* is used. For minor cuts, burns and for inflammation, it is used externally. In children with upper respiratory, eye and ear infection, the leaf juice is mixed with honey. In the treatment of cirrhosis of the liver and infectious hepatitis, *E. alba* is used in the phytopharmaceutical formulations of medicines [24]. For rattlesnake bite, wedelolactone obtained from this plant is used. The shoot extract shows antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*.

3.4 Antiviral activity of *Eclipta alba*

Against many viruses, the extracts of *Eclipta alba* were proved to have strong antiviral activity [25]. Though the fresh juice of leaves is used for various benefits like increasing appetite, mild bowel regulator, it is mainly used in viruses causing blood borne hepatitis. It is also used to protect the parenchyma and to promote bile flow which is popularly used to enhance learning and memory. In most parts of the subcontinent, it is used for jaundice, as a deobstruent and cholagogue in hepatic injury and other ailments of liver. One of the most potent inhibitory activity against HIV-1 integrase [HIV-1 IN] was found in the water extract of *Eclipta prostrate* [26].

4. *Phyllanthus amarus*

In Ayurveda, *Phyllanthus amarus* is an Indian herb that has been used in support liver. 'Phyllanthus' is also represented by the name "leaf and flower" as the fruit and the flower are found under the leaf [27].

4.1 Origin and distribution

In all tropical regions of the planet, *P. amarus* is widely distributed. The exact geographic origin of this plant was not found by Paleobotanical studies [28, 29]. This plant may be indigenous to the tropical Americas and also to the Philippines or India [22].

Taxonomy

Kingdom	-	Plantae
Division	-	Angiospermae
Class	-	Dicotyledoneae
Order	-	Tubiflorae
Family	-	Euphorbiaceae
Genus	-	*Phyllanthus*
Species	-	*amarus*
Binomial name	-	*Phyllanthus amarus*

Floral formula

Br, Ebrl, p[5], A5 G[3]

4.2 Chemical constituents

Some of the secondary metabolites that are present in *P. amarus* include lignins alkaloids, hydrolysable tannins, flavanoids, and polyphenols. Such phytochemicals and their structure were usually determined by using IR, UV, NMR and mass spectroscopy [30]. Apart from that, preparative thin layer chromatography and column chromatography are also used by which few new alkaloids were isolated [31].

4.3 Pharmacology

Parts of this plant *P. amarus*, mostly fresh and occasionally dry are sold and the decoctions are used widely for herbal baths. To treat bladder and kidney disorders, herbal decoction of this plant is used in traditional medicine [32]. This herb can also be used as tonic and can also be used as an appetizer. *P. amarus* plant extracts is also used as blood purifiers. It also helps to release phlegm for light malaria fevers and anemia [33]. In combination with many other herbs, this herb is used for curing flu, asthma and fever. The leaves are boiled and used to treat dysentery and even skin disorders [34]. For constipation also, studies have proved that extracts are very effective. Apart from that, extracts have even shown anti-cancer properties. It also has beneficial effects on liver functions.

Several studies in the past have proved that *P. amarus* inhibits the activity of the HBsAg [35]. Widespread studies on *P. amarus* have showed that the extracts possess strong antiviral activities particularly against HBV and HCV. In Indian system of medicine, a chemo-biological fingerprinting methodology for standardization of *P. amarus* preparation was even patented [36].

4.4 Antiviral activity of *Phyllanthus amarus*

Evaluation of antiviral activity of *Phyllanthus* species was evident from aqueous extract showing strong activity against viruses like HSV1 and HSV2 in vero cells by quantitative polymerase chain reaction. To study protein expressions of treated and untreated infected vero cells, western blot and 2D-gel electrophoresis were most widely used. *Phyllanthus amarus* along with *Phyllanthus urinaria* demonstrate the strongest antiviral activity against both HSV1 and HSV2 viruses which is proposed to its action in the early stage of replication and infection [37].

In conclusion, in the existing scenario, there is an essential need for the development of new antiviral drugs. As on date there are many epidemiological studies that have evaluated different targets of these antivirals and promising results were obtained. Still a lot of research is needed to find out the actual potential for human use.

Ethnobotanical information				
Plant	Parts used	Benefits	Information obtained from	Reference
Boerhavia diffusa	Roots	In the treatment of piles. Root paste is used to cure bloody dysentery. It is also used for leukorrhea, rheumatism and stomach ache. The root juice is used in treating asthma, scanty urine and internal inflammation disorders.	India	Mitra and Gupta [38]
Eclipta alba	Flowers, leaf and roots	Remedy for catarrh in infants, toothache, headache and roots are emetic and purgative	India	Puri [39]
	Entire plant	Remedy for the treatment of bleeding, hemoptysis, hematuria and itching, hepatitis, diphtheria and diarrhea	Taiwan	
	Entire plant	As a cooling and restorative herb, which supports the mind, nerves, liver and eyes	China	
Phyllanthus amarus	Aerial parts of the plant	Used in the treatment of liver, kidney and bladder problems. Also used for diabetes and intestinal parasites	Many parts of the world	Chevallier [22]
	Entire plant	Kidney stones	Spain	Houghton et al. [31]
	Entire plant	Used to eliminate gall bladder and kidney stones, and to treat gall bladder infections	South America	
	Entire plant	Used in traditional medicine to treat liver diseases, asthma and bronchial infections	India	Foo [30]

In comparison to synthetic counterparts, extracts obtained from *Boerhavia diffusa*, *Eclipta alba* and *Phyllanthus amarus* are tend to be safer, very effective and cheap. The extracts obtained from these plants might have compounds that are true antiviral, but are present at insufficient quantity to inactivate all infectious virus particles. It is very much possible that obtaining active constituents from these plants will be providing many useful leads in the development of effective antiviral agents. Thus there is an urgent need to find effective antiviral agents as the existing drugs may be effective but in a specific manner. Based on the existing knowledge of traditional medicine, these three plants have to be explored further for formulating effective drugs against various dreadful disease caused by viruses.

Author details

Ganesh Kumar Anbazhagan[1]*, Sankarganesh Palaniyandi[1,2]* and Baby Joseph[2]

1 Department of Microbiology, Hindustan College of Arts and Science, Chennai, India

2 Centre for Research and Consultancy, Department of Food Technology, Hindustan Institute of Technology and Science, Padur, Chennai, India

*Address all correspondence to: ganethkumar@gmail.com and bilisankar@gmail.com

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Medicinal plants are a source of a wide variety of biologically active compounds that have been used extensively over many centuries as crude material or pure compounds for treating various diseases. Of the estimated 250,000–500,000 species of plants on Earth, only 1%–10% are used by humans. Plant-based traditional medicine plays a key role in the development and advancement of modern studies by serving as a starting point for the development of novelties in drug discovery. This book presents a comprehensive overview of medicinal plants and their potential uses, with chapters on Chinese traditional medicine, antioxidant activity of faba bean extracts, and antiviral activity of several plant extracts. This book will prove useful to a wide variety of readers, from medical professionals to farmers and gardeners to those interested in herbs and herbal medicine.

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