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A Great Challenge on The Reproducibility of Therapeutics Results of Phytopharmaceuticals

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Abstract
Phytopharmaceuticals contain a complex mixture of constituents from herbal medicine. It is important to understand that phytopharmaceutical therapeutic results and safety are built from all its constituents. Reproducibility of phytopharmaceuticals quality must be guaranteed from raw material to end product and from batch to batch production. Determination of quality cannot only be based on one or group of marker compounds because it will not be able to describe a complex mixture of ingredients. To represent the complexity of constituent in phytopharmaceuticals, chemical fingerprints have to be used which can be obtained by various chromatography and other methods.

Keyword: phytopharmaceuticals, complex mixture, marker constituent, chemical fingerprint, medicine, R&D investment.

1 Introduction

For many centuries, herbal medicine has been used to prevent and cure of various diseases. This relates to cultural popularity, ease of access, and relatively low prices. In ancient times, herbs use was based on instinct, taste, and experience, so that people could distinguish plants that were beneficial to health, not useful or toxic. The public can also find out which processing method should be used to obtain optimal efficacy[1] Chinese, Unani, Homeopathy, and Siddha. Traditional medicine using herbal formulas is widely used to treat complex diseases for years. The herbal formula used often consists of several herbs and contains various chemical compounds, making it possible to have several unknown targets and treatment functions[2]. Medicinal plants are also important sources of substances with biological activity. Approximately 25% of modern medicines are directly or indirectly derived from plants [1].

Herbal products have excellent potential in the pharmaceutical segment. There are three categories of herbal products; traditional herbal medicine, over-the-counter (OTC) herbal medicine, and phytopharmaceuticals. Traditional herbal medicines are based on empirical efficacy and supported by ancient beliefs, and prescribed individually. The OTC herbal medicinal products do not yet have scientific clinical data and evidence but are already in pharmaceutical preparations. The phytopharmaceuticals made based on data, scientific evidence, and clinically tested[3]. The phytopharmaceuticals is a standardized herbal preparation containing active ingredients in a complex mixture of plant ingredients. This preparation does not have an immediate or strong pharmacological effect, therefore, it is not appropriate for emergency treatment [1][4].

An herbal product, either in the form of plant parts (fresh or dried) or plant extracts, contains many constituents that often work together synergistically. The active ingredient in herbal medicine preparations is the entire chemical content. It can also be one of the compounds that have confirmed therapeutic activity or group of compounds with the same core structure and are generally accepted that have a substantial contribution to the drug's therapeutic activity. With varying chemical contents in a plant, no one plant is recommended for only one disease; and no one disease is related to only one plant [1][4][5].

Besides having tremendous potential, various reports show the side effects of herbal products. People always assume that herbal medicines are safe and there are no side effects compared to synthetic drugs due to its nature. This perception causes herbal medicines are often misused and consumed like food, not as medicine. Various studies have shown there are many interactions between herbs with herbs or herbs with drugs. The herbal medicines are considered as complementary medicines that can be consumed together with synthetic medicines [6][7].

Even though herbal medicines lately tend to increase, unfortunately, it is followed by an increase in various adverse effects of herbal use. One of the biggest causes of these cases is closely related to the poor quality of phytopharmaceuticals used. For this reason, the issue of phytopharmaceuticals quality is crucial to consider [8].

Difficulties in quality control and lack of scientific evidence in term of herbal medicines efficacy have resulted in their use being replaced by a synthetic drug, in alternative medicine, herbal medicine still exist in various parts of the world [4][5]. For this reason, the chemical substance composition in phytopharmaceutical has to fulfil the quality because it will influence effectiveness and safety's reproducibility at the clinical level [5][9].

2 Common challenges in phytopharmaceuticals

Herbal medicines usually consist of plant parts, either single or in combination in pharmaceutical dosage forms. Like synthetic drugs, herbal medicine also has therapeutic or prophylactic properties. The interaction of its multi-component ingredients causes poly-pharmacological effects or is known as a multi-target effect. The application of this concept in phytopharmaceuticals development requires comprehensive characterization of chemical content and bioactivity [10].

Phytopharmaceuticals can become part of the health care system if it can prove their quality guarantee the safety and efficacy as expected. But until now, it is still challenging to ensure the quality, safety, and effectiveness of phytopharmaceutical. Quality assurance must be applied at every production stage starting from cultivation, harvesting process, post-harvest processing, manufacturing, packaging, and product distribution. Quality assurance at each step is intended to ensure the reproducibility of its effectiveness and safety [5][9][11][12].

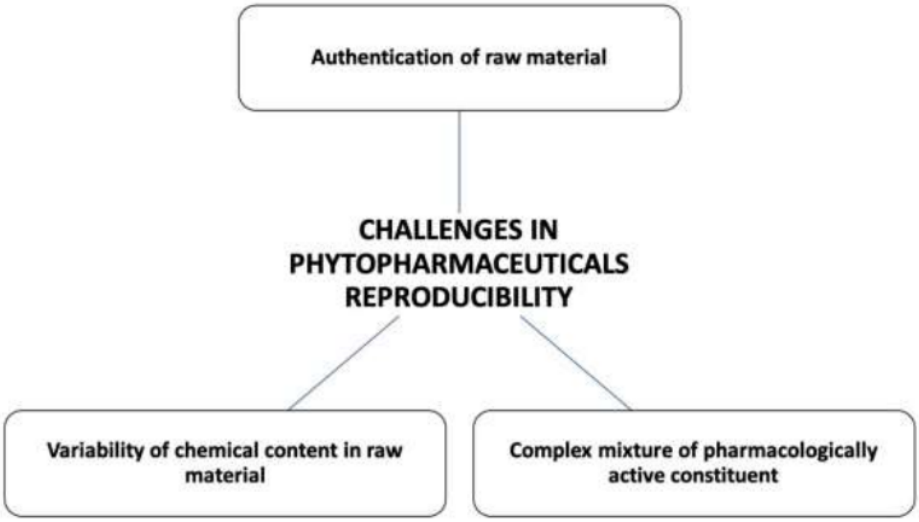

Figure 1. The great challenges in pharmaceuticals reproducibility

In the following, we will discuss the three most significant challenges of developing phytopharmaceutical products not found in synthetic drugs as illustrated in Figure 1.

2.1 Authentication of raw material

Lately, the use of phytopharmaceuticals has increased so that medicinal plants have become an important agricultural commodity. Many medicinal plants are sold in various dosage forms such as fresh ingredients, dried ingredients, dry powdered materials, and extracts, making it impossible to authenticate morphology because such materials have lost their characteristics. Increased demand for medicinal plants has caused unethical commercial trade, such as replacing authentic raw materials with other materials that are similar but less effective. The act of counterfeiting or mixing herbs of low-quality herbs will affect consumer health and safety so that authentication and determination of the quality of herbal raw materials is a critical point in guaranteeing the quality of pharmaceuticals [13][14].

Authentication is a guarantee of compliance with the required specifications. Authentication covers various aspects, including identifying, quantifiying of characteristic components, adulteration, contaminants, and verification of quality according to specifications. Specification of medicinal plant raw material is including botanical or geographical origin, manufacturing, or processing procedures of its preparation [15][16].

Ancient healers have a very high ability to recognize the ingredients used in their medicinal herbs. However, with the development of modernization, the loss of contact with nature have reduced the ability to identify medicinal plants. Ancient texts about herbal medicine are often translated and interpreted differently, and as a result, there are the same names for more than one plant and different names for one plant. This condition is increasingly tricky with language diversity and local dialects [17][18].

The morphological similarity of plant parts causes misidentification of herbal raw materials. Faulty raw materials can occur either intentionally or unintentionally.

Adulteration usually occurs concerning the material's economic value, for example, by replacing authentic raw material with other similar plant species. Accidental mistakes can happen mainly because workers' carelessness collect wild plants as raw material [19–21].

Nomenclature problems cause misidentification that often occurs. Local names of plants are often referred to for their traditional use. The local name also often causes mistakes in raw materials and becomes dangerous, especially when replaced by poisonous plants. For example, in Traditional Chinese Medicine (TCM) the name "Fangji" is used for the root of *Stephania tetrandra* S. Moore (Menispermaceae). This species is originated from the northern part of China. The official name in Chinese Pharmacopoeia is "Hanfangji". The name "Fangji" is also used for the roots of *Aristolochia fangchi* YC Wu ex LD Chow & SM Hwang (Aristolochiaceae) originating from the southern region. In pharmacopeia referred to as "Guangfangji", and contains nephrotoxic and carcinogenic acid derivatives [11].

Misidentification also occurs in different species of plants that have the same local name. The two species have different pharmacological activities so that their use will not achieving therapeutic targets. In Indonesia, *Mesua ferrea* is called "Nagasari" or in India called "Nagakeshara", an herb used for heart tonic, cardiotonic, emenagogue, hypotensive, antispasmodic, antianaphylactic, and antiasthmatic. However, *Calophyllumino phyllum* is also sold under the local name "Nagasari "or "Nagakeshara". This plant is usually used for antiinflammatory and treatment of psoriasis [22][23].

Hypericum androsaemum is traditionally used for its diuretics and hepatoprotectives. Due to high demand but not in abundant stock, the raw material of *H. androsaemum* on the market is often found mixed with other genera which more abundant and cheap or sometimes accidentally mixed with *Hypericum perforatum*. The traditional medicinal ingredient in China for leg pain and anti-inflammation, *Piper kadsura*, is often replaced by *Piper wallichii*. Both species have morphological similarities but *P. wallichii* has no medicinal value. The same case also occurs with oriental ginseng (*Panax ginseng* C.A. Meyer) which is also often mixed or replaced with American ginseng (*Panax quinquefolius*). Traders are forced or deliberately replace the plant parts of the same species or replace them with other species, which have a lower pharmacological or not at all [24]–[27].

Misidentification cases also occurredin black cohosh plant (*Actaea racemosa* L., Ranunculaceae). This plant is a raw material for menopause therapy products. This product causes hepatotoxicity ini consumers. Valid analysis methods can prove that products on the market do not contain the right plants [21][28].

In traditional Chinese medicine, *Marsdenia tenacissima* (Roxb.) Wight et Arn. (Asclepiadaceae) used to treat esophageal, gastric, lung, and liver cancer. The *M. tenacissima* is known by the traditional name as "Tong-guanteng" and is sold in dried, sliced, shredded, and processed forms so that it is often difficult to identify the plant. The *M. tenacissima* is often replaced with *Telosma cordata* (Asclepiadaceae) and *Fissistigma polyanthum* (Annonaceae) which cause a poor therapeutic effect and even cause unwanted effects [14].

2.2 Variability of chemical content in raw material

Side effects of herbal product usage have been widely reported. These incidents are caused by mistaken authentication, adulteration, contamination by a microorganism or toxic chemicals, overdose, improper use, and interaction with drugs. There are still many raw materials of medicinal plants obtained from wild populations. Whether

intentional or unintentional, mistake often occur in the harvesting process, causing raw material to be contaminated by other species or unneeded plant parts. The poor quality of finished products is caused by the use of raw materials of phytopharmaceuticals that do not have high enough quality standards. The quality of raw materials of medicinal plants is greatly influenced by various factors such as intrinsic (genetic) and extrinsic (environmental) factors during their growth, cultivation, harvesting, post-harvest process, transportation, and storage [8].

2.2.1 Intrinsic Factor

Plants synthesize secondary metabolites such as phytoprotein and phytoalexin to defend themselves from environmental conditions involving insects, plant microorganisms, and other plants. The secondary metabolites in plants can be manipulated using genetic engineering, to increase desirable the production and reduce the undesirable compounds. The synthesis of flavonoids and anthocyanins is the first genetic engineering that has been successfully carried out, because the biosynthetic pathway is well known. The results can be detected from changes in flower color. The indole terpenoid alkaloids pathway is an attractive target for genetic engineering because about 15 terpenoid, alkaloids, and indole terpenoid alkaloids have an essential role in anti-tumor alkaloids, vinblastin, vincristine, and camptothecin. The success of genetic engineering in the future achievement of the pharmaceutical industry is used for the expansion of new compounds with new activities. But in some cases, excessive gene expression occurs so that it does not reach the desired target compound [29].

2.2.2 Extrinsic Factor

The environment and habitat are external factors that affect the types, proportions, and levels of active substances of the widespread species. Biosynthesis of the active substances in a plant result of interactions between plants and the environment in a long evolutionary process. The research on *Eucommia ulmoides* Oliv. reported that the height of the location and the average annual temperature were highly significant with chlorogenic acid and flavonoids levels. The duration of yearly sunlight positively correlated with geniposidic acid but negatively correlated with geniposidic acid levels. *Potentilla fruticosa* L. in traditional Chinese medicine is used for detoxification and for treating diarrhea, hepatitis, rheuma, and scabies. The *P. fruticosa* grows in cool and high regions in the northern subarctic mountains. Liu et al proved that altitude has negatively correlated with tannin content, whereas the length of annual sunlight and altitude has positively correlated with flavonoid levels and antioxidant activity. The average yearly temperature has negatively correlated with total phenolic levels, while altitude has positively correlated with total phenolic levels [30].

Ecological factors such as climate, geography, land, and topography can affect plant's growth, development, reproduction, behavior, and distribution. To overcome these effects, plants can regulate the synthesis of secondary metabolites. Different ecological factors cause differences in plant quality when compared to the original area. *Dendrobium officinale*, one of the traditional Chinese medicinal plants, is used as a tonic, nourish the stomach, relieve throat inflammation, improve eyesight, and promote body fluid. The main ingredients of *D. officinale* are polysaccharides, alkaloids, and flavonoids. To meet market demand and keep the original plant, the study results indicate that the this plant's cultivation must fulfil the factors such as humidity, temperature, duration of sunlight, soil pH, nitrogen and phosphorus content in the soil needed by the plant [31].

Plant interaction with their environment causes variations certain secondary metabolites "classes'' composition and production. The composition of constituents can

be used for characterization as chemical markers for plants that grow from certain geographical areas, which are harvested at certain seasons or certain ages based on qualitative and quantitative analysis of its chemical constituents. *Tithonia diversifolia* (Hemsl.) A. Gray, belonging to the Asteraceae family, commonly known as Mexican sunflower, produces essential oils that spread worlwided. This species is native to Mexico and Central America and is a weed that spreads in various ecosystems, especially in Africa and China. The results of Sampaio and Da costa's research showed that there are different chemotypes for *T. diversifolia* species according to the sample's geographical origin. Differences in the accumulation of ß-pinene in essential oils and variations in the production of certain classes of secondary metabolites, such as terpenes, seems to response to different abiotic environmental conditions directly. This study also shows that tissue-specific changes that produce secondary metabolism are adaptive strategies for different environments [32].

2.2.3 Harvesting

Determination of harvest time is critical to get quality raw materials. Post-harvest processes such as processing into dry raw materials, transportation, and storage must also be carried out at optimal conditions to produce the reproducible quality of raw materials. The primary source of medicinal plants comes from the wild, so that exploitation can cause natural damage and extinction of some species. Plant collected from the wild have problems regarding the homogeneity of the quality. The agroclimatic conditions will affect the chemical composition and therapeutic properties. One medicinal plant widely used in India, *Terminalia chebula*, originates from India's various parts, known to have different therapeutic properties [33].

Harvest time will affect the composition of the chemical content in plants. Therefore the harvest must be done at the right time. It is known that the chemical content of plants is directly correlated to the stage of plant development. In Ayurveda, *Andrographis paniculata* (Kalmegh) used as a hepatoprotective. A study conducted by Pandey and Mandal revealed that the maximum andrographolide levels (2.85%) were found at the initiation of flowering or harvested after 130-150 planting days. The highest level of Rauwolfia serpentine root alkaloid is at the age of 18 months. Plant maturation stage at the root of *Wihania somnifera* (Ashwagandha) is 130-180 days after planting, while the peak maturity of *Tinospora cordifolia* (Giloe) stems is 15 months. In Ayurvedha, *Adhatoda vasica* (Adusa or Vasaca), used as a bronchodilator contains vasicine on its leaves. This plant blooms in March (fully bloomed) and September (partially blooms). At the flowering stage, levels of these compounds were 3.0 and 1.4% in March and September, respectively. Whereas at the vegetative stage, the vasicine levels are very low [33].

Lemongrass oil, obtained from (*Cymbopogon citratus*), is one of the essential oils that is widely used in the perfume, cosmetic, beverage, and pharmaceutical industries as aromatic and antiseptic. Citral content is a characteristic of lemongrass oil quality. There are 65 compounds in lemongrass oil, but only 13 compounds are always present in the lemongrass oil harvested at different ages. Citral levels are also different when lemongrass is harvested at different maturity stages. Optimal citral content in lemongrass oil is obtained when harvested at the age of 6.5 and 7.0 months [34][35].

Artemisia annua L. (Asteraceae) which has antimalarial activity, is a commercial antimalarial compound sources. Essential oils of *A. annua* are used in the perfume and cosmetics industries. In the essential oil is found main compounds such as camphor,

artemisia ketone, germacrene D, and 1,8-cineole. The variability of the chemical composition of essential oils of *A. annus* depends on the geographical origin and stage of plant development. Before the flowering stage, the essential oil content was 0.8%, then increased rapidly to 1.38% after the flowering stage. The main compounds (artemisia ketone, camphor, and 1,8-cineole) and the highest antioxidant activity in essential oils are taken at the full flowering stage [36].

Adulteration that occurs is often done for profit. Adulteration is done by adding foreign material, which is usually cheaper and dangerous. *Illicium verum* Hook.F., which is used as a drug for infant colic, is often counterfeited or mixed with *I. anisatum*. *I. anisatum* contains neacoxic sesquiterpene dilactone anisatin, a strong non-competitive GABA antagonist. Both have morphological similarities. This adulteration case causes many cases of adverse neurological reactions such as nausea, hallucinations, and epileptic seizures, especially in small infants [11].

Another example of adulteration occurs in Saffron (dry red stigmata *Crocus sativus* L.), one of the expensive raw materials. Saffron is often added with other similar ingredients, reducing the therapeutic efficacy. Other raw materials such as ginseng are often diluted with cheap fake ingredients *Platycodon grandiflorus* (Jacq.) A. DC. or with different ginseng species, such as *Panax quinquefolius* L. (American ginseng), *P. ginseng* C.A. Meyer (Asian ginseng), or *P. notoginseng*. There is a problem with this mixing. Different ginseng species have different saponin profiles, thus giving different pharmacological effects. American ginseng and Asian ginseng have been reported to have contradictory effects on the vascular system and blood glucose levels [11].

2.3.4 Post-harvesting process

Water is a component that determines the physical and chemical properties of plant constituents. Decreasing plants 's moisture content of by the drying process, intended to prevent the activity of enzymes and microbes, extend shelf life. Dry raw materials simplify the process of transportation and storage. Many factors affect the drying method. Therefore a drying method must be chosen that does not change or reduce the phytochemical content [11][37].

Camellia sinensis or *C.assamica* is the most popular drink in the world whose use can be in the form of green, black, or oolong. Green tea has important and extensive pharmacological activities. But to maintain its quality, the drying method is very limited due to the instability of its chemical content. Chemical ingredients in green tea are vitamins, chlorophyll, flavonoids, dan polyphenols which have antioxidant activity [38].

The drying process affects the recovery of taxol from *Taxus baccata*. Yield taxol from the stem is not affected by drying temperature, while the yield of taxol from leaves will increase with increasing drying temperature. Drying at low temperatures will produce a low yield because it requires a longer drying time so that the enzyme activity causes taxol to degrade [37].

2.3.5 Storage

After post-harvesting, medicinal plants often have to be stored long enough before being used as raw materials for various products. For this reason, the storage method must be suitable so that it does not cause physical, chemical, and microbiological changes. Storage is intended to ensure that there is no loss of quality. Some methods are used such as control humidity and air movement to prevent metabolic activities, which can damage the content of compounds in it. Another way is to prevent attacks by insects, mice, and microorganisms [39][40].

Echinacea purpurea (Asteraceae) is a medicinal plant that has immunostimulatory and anti-inflammatory activity, especially in alleviating cold symptoms. The storage process affects the content of alkamide and cichoric acid in *E. purpurea* root, in reverse. Alkamide levels did not change when stored for 60 days in the dark at 5^0C, while cichoric acid levels reduced by 70%. On storage in light at 20^0C, the cichoric acid level did not change, while the alkamide level dropped by 65%. Temperature and light affect the compound content of *E. purpurea* [39][41].

The composition of the chemical content of essential oils from aerial parts of *Thymus daenensis* Celak did not show changes during storage in the freezer (-20°C). Storage at room temperature (25°C) also does not reduce its quality because there is an increase in the levels of thymol and carvacrol. The storage temperature affects the composition of compounds in the essential oils of *Hyptis pectinata* L. Poit. Compared with storage in the freezer, storage at room temperature showed increased levels of β-elements, α-copaene, germacrene D, and caryophyllene compounds. Oxide and (E, E) - α-farnesene and decreased levels of α-humulene and β-caryophyllene, compared to storage in the freezer [40].

Changes in chemical composition that occur in the storage process will cause biological activity changes. Nine medicinal plants from Africa showed a loss of anti-inflammatory activity (inhibition of COX-1), while the antibacterial activity did not change [42].

The type of packaging used in the storage process will also affect the chemical content of the stored medicinal plants' raw materials. Various studies show that the packaging used dramatically influences compounds' content and composition in essential oils, polyphenol group stability, and color stability [40].

2.3 Complex mixture of the pharmacologically active constituent

The plant synthesizes primary metabolites for normal growth, development, and reproduction for its original life. Primary metabolites such as sugar, protein, vitamins, lipids, and starches are needed for growth and development, whereas chlorophyll, amino acids, nucleotides, and carbohydrates needed for metabolic processes such as photosynthesis, respiration and nutrient assimilation. However, to protect themselves from potential dangers from environmental conditions or other species, plants will synthesize secondary metabolites. This secondary metabolite is synthesized from the substrate used for the primary metabolite or modification of the synthetic pathway from the primary metabolite. Secondary metabolites are not directly needed for growth and will accumulate during growth. This compound has biological activity, so it has the potential for humans to be used as a medicine [43][44].

The complexity of the chemical content makes it challenging to control herbs' quality. Therefore, the development of new drugs is preferable to use isolates of active compounds obtained from the separation and purification process guided by bioassays. In 1897 aspirin, which is synthesized from salicylic acid extracted from willow bark causes an era of concept dominance mono-drug therapy and the development of synthetic drugs and causes a decrease in the use of natural products drug discovery [45].

Various studies have shown that the separation and purification process reduced or even reduced its pharmacological activities. The antimalarial activity of artemisinin is lower than the whole extract of Artemusua annua L. This decrease in effect is strongly related to the loss of pharmacokinetic synergy between constituents after herbal extracts have been purified. The decreased activity also occurred in the process of isolating Aconiti (*Aconitum carmichaelii* Debx), gentiopicroside (*Gentiana manshurica* Kitag), liquiritigenin and isoliquiritigenin (*Glycyrrhiza uralensis* Fisch), ginsenoside Re (*Panax*

ginseng C. A. Mey), cryptotanshinone and tanshinone (*Salvia miltiorrhiza* Bge), schizandrin (*Schisandra chinensis* (Turcz.)). The pharmacokinetic synergy effect between constituents in herbal extracts can occur during absorption, distribution, metabolism, and excretion [45][46].

The content of 5'-methoxyhydnocarpin, a strong P-gp inhibitor, increases cellular absorption with substrates p-glycoprotein (P-gp). Both of these compounds are found in *Berberis* plants. Arteannuin B, one of the compounds in *Artemisia annua* extract, can increase the AUC0 – t (2.1-fold) and peak concentration (Cmax, 1.9-fold) of oral artemisinin in mice [46]. Radix *Polygoni multiflorum* Tunb. contains 2 main components, stilbene glucoside, and emodin. Stilbene glucoside can increase the absorption and duration of action of emodin by inhibiting UDP-glucuronosyl-transferases 1A8. Thus it was inhibiting emodin glucuronidation [47].

The flower of *Abelmoschus manihot* (Linn.) contains flavonoids such as rutine, hyperoside, isoquercitrin, hibifolin, myricetin, quercetin-3′-Oglucose, quercetin. This flowers' decoction is traditionally used to the treat chronic renal disease, oral ulcers, and burn in China. This decoction also contains nonflavonoids of small molecule ingredients such as organic acids, amino acids, nucleosides, oligosaccharides, and non-flavonoid macromolecule fractions such as pigments, resins, polysaccharides. Various study showed that the bioavailability and elimination of flavonoid compounds were influenced by non-flavonoid compounds [48].

The compound contents' complexity and the synergistic pharmacokinetics that occur naturally in herbs is a high-efficiency natural drug delivery system in herbal extracts. This pharmacokinetic synergy can through various mechanisms including by increasing solubility, preventing enzymes from metabolizing drugs, eliminating first-pass mediated efflux-drug-transporters, increasing membrane permeability, opening tightly crossing paracellular cells, and changing the shape and absorption of bioactive compounds (for example, by forming efflux-drug-transporter-mediated naturally occurring nanoscale particles) [46].

Unlike synthetic drugs, phytopharmaceutical contains many pharmacologically active components. The complex constituent composition of phytopharmaceutical is an advantage because various compounds can produce additive or even synergistic effects or can also work multifactorial, which involves many pathways from target therapy. Interactions between chemical substances can also increase solubility and bioavailability simultaneously. However, its complex chemical composition is also an obstacle that must be faced to make it reproducible. The concentration of chemical composition can vary from batch to batch. This variation in the chemical composition will affect its effectiveness [11][49][50].

3 Strategy to guarantee the quality of phytopharmaceutical

In the past, the use of herbal products was done by individual healers. In this case, the healer will be the only one in determining the quality of raw materials and their production. The success or failure of therapy are also primarily determined by the patient's trust in the healer. The absence of reproducibility of therapy is unacceptable in phytopharmaceuticals products. Reproducibility of product quality and the guarantee of effectiveness and safety are very important. The most important thing to ensure the quality, from raw materials to finished products, is to develop the suitable quality control method to be able to guarantee its quality [51].

Quality assurance begins with standardization. Standardization is not only analytical operations that only determine the identity and level of active compounds in phytopharmaceuticals. Standardization is an activity that establishes the complete

information and control needed to ensure the consistency of the composition of the constituent of phytopharmaceuticals. The use of analytical methods must pay attention to the fact that plant material has a constituents' complex composition. It also must understand the variability and inconsistency of the composition influenced by many factors that cannot be eliminated. These conditions complicate the standardization of phytopharmaceuticals, so the application of analytical methods for quality control requires the latest innovations and techniques [52][53].

Quality control is a process involved in maintaining the quality and validity of manufacture. In general, quality control is based on three aspects, Identity, Purity, and Content. Identity mainly concerns species authentication and all characteristics that are under the specifications of phytopharmaceuticals raw materials. Its characteristics include macroscopic and microscopic tests, organoleptic tests such as colour, smell, and taste. The purity of phytopharmaceuticals raw materials is related to the uses' safety. Purity is related to ash value, contaminants (microorganisms, heavy metals, pesticides), and foreign matter. The application of the latest analytical methods can measure aflatoxin, radioactivity, and extracting solvent residues. Content or assay is the most challenging aspect to do, considering the variability and complexity of phytopharmaceuticals' chemical content. This aspect also includes the determination of loss on drying, moisture content, and essential oil content. The chemical constituents' determination in phytopharmaceuticals is quite tricky because in most herbal medicines the active constituents are unknown. The pharmacological activity of phytopharmaceutical comes from all the compounds in it. The concept of marker can be used to determine quality but cannot describe the reproducibility of the whole component. For this reason, the concept of a metabolite profile or metabolite fingerprinting must be used [1][51][54].

It is well understood that like all herbal products, phytopharmaceuticals contain multicomponent. The multicomponent composition is not yet known and is not clearly understood. The complexity of phytopharmaceuticals' chemical content requires different concepts in quality assurance. The approach that can be used today is compound-oriented or pattern-oriented. The compound-oriented approach uses certain components with some chemical properties known as targets while pattern-oriented uses all components that can be detected as targets. The compound-oriented approach is based on a marker compound concept with the target is certain chemical compounds with known molecular structures. A pattern-oriented approach on a metabolite profile or fingerprinting or phytoequivalent concept with a target is all chemical compounds with structures that are known or unknown or that have partial chemical information eg. retention time, mass spectrum, and ultraviolet spectrum [55]–[57].

3.1. Marker compound concept

In phytopharmaceuticals, the resulting pharmacological activity comes from all the compounds in it. The active compound cannot be ascertained. Besides, the ingredients are very complex and varied so that it becomes more challenging to characterize. Under these conditions, a marker is needed to represent phytopharmaceuticals' characterization. These marker compounds are used to control quality but do not always represent the constituents associated with drug activity [1][54][58].

In pharmacopeia, phytopharmaceuticals are considered drugs so that the quality is based on a chemical marker, which is expected to ensure consistency. According to the European Medicines Agency (EMEA), chemical marker are constituents or constituent groups used for quality control purposes, have or do not have therapeutic activity. To be a marker of quality, ideally, a chemical marker has unique and specific characteristics and must contribution to the therapeutic effect of phytopharmaceuticals. Differences in the

number of chemical markers can indicate differences in the phytopharmaceuticals pharmacological activity. Thus, the number of chemical markers can an indicate the quality of phytopharmaceuticals [59][60].

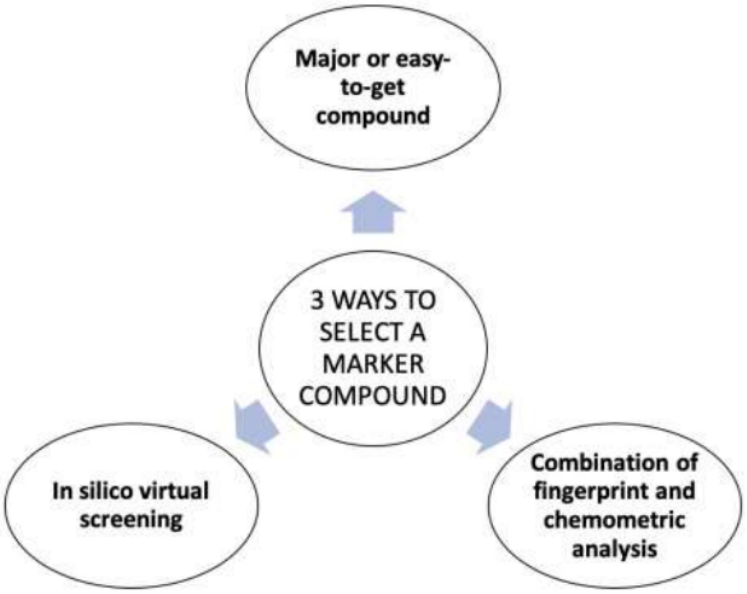

Figure 2. Three ways to select a marker compound

In the concept of marker compounds, the key first step in determining phytopharmaceuticals quality is the selection of marker compounds. There are three ways to choose or select a marker compound that will be discussed below as illustrated in figure 2.

- Major or easy-to-get compound

  The selection of marker compounds isn't too easy because the therapeutic or bioactive components of phytopharmaceuticals are unknown and not yet clearly understood. Under such conditions, the marker compound can be selected from several components that are not specific but easily obtained. But, this has a weakness, the marker compound cannot ensure the effectiveness of phytopharmaceuticals [60]. This easy-to-get compound is usually a major compound. At least, because the levels of these compounds are large, they also have a major contribution to phytopharmaceuticals' pharmacological activity.

  An example of this case is ethyl-p-methoxycinnamate as a marker compound in the ethanolic extract of *Kaempferia galanga* rhizome [61]. The compound content is 14.54%. In the ethanolic extract of *Andrographis paniculata*, the Andrographolide compound is 10.82%. This compound is used as a marker compound for products containing *A. paniculate* extract [62]. However, various research showed that the pharmacological activity of the extract is greater than its marker compound. This indicates that selected marker compound is not therapeutically active, but the levels

can be a marker quality of their pharmacological effectiveness. These compounds can be called active marker compounds.

- In silico Virtual Screening

  Marker compounds must have clear pharmacological activities so thattheir levels in the product are an indication of product quality. Bioassay screening is indispensable to obtain such compounds. The next process is to carry out bioassay-guided isolation. Then proceed with bioactivity confirmation using animal models, organ and tissue models, cellular models, or receptors and enzymatic. However, this method is time consuming, difficult, and has not revealed the effects of interactions with other compounds. Therefor requires an integrated new approach for predicting potential active constituents in herbs [60].

  Recent developments in efforts to explain the pharmacological basis of medicinal plant functions have been carried out using in silico approaches such as virtual screening and tissue analysis. This approach can increase the discovery of active compounds and can show the mechanism of action of medicinal plants, reduce costs, and improve the whole procedures' efficiency. Some stages in the in silico approach are the target selection of medicinal plants, target plants' database, ADME/T analysis (absorption, distribution, metabolism, excretion, toxicity), and in silico virtual screening. In silico virtual screening method is carried out with the following steps: virtual targets based on pharmacophore theory; double validation based on the theory of similarity in the form of small molecules; and analysis of target compounds based on docking. The initial half of the implementation of this method uses software technology, including the prediction of multiple activities and virtual screening. All are based on mass spectra data to provide accuracy. Compared to conventional screening methods, the in silico virtual screening method is superior because it is only required information on the structure of compounds. This method can also multi-target screening a large number of compounds in a relatively short period, thereby reducing costs and time [63].

  The herbal formula for tuberculosis therapy in Indonesia uses several medicinal plants such as *Curcuma xanthorrhiza* Roxb., *Tamarindus indica* L., *Citrus aurantifolia, and Zingiber officinale var. rubrum*. The results of tests using in silico virtual screening successfully revealed 6 compounds namely curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid which are related to their activities in inhibiting cell growth and MTB (*Mycobacterium tuberculosis*) infection. These studies indicate that curcumin, gingerol, phytol can be used as a marker compound for *C. xanthorrhiza* Roxb., *Z. officinale var rubrum, Tamarindus indica* L., respectively [64].

- Combination of fingerprint and chemometric analysis

  A fingerprint is a method that describes the characteristics of complex samples obtained from integrated chromatography or spectroscopy. The chemometric analysis is used to correlate chemical profile data obtained from the fingerprint method with pharmacological profile data from complex samples. Chemometric analysis with the help of mathematical, statistical, and computational sciences such as PCA, PLS-DA, hierarchical cluster analysis (HCA), K-nearest neighbour (KNN), and so on, can be used to obtain marker compound information in medicinal plants. The results of the correlation will show compounds that have certain activities. Using chemometric analysis, the HPLC profile data of *L. japonica* samples were correlated with bacteriostatic data. The results showed that 6 constituents are positively correlated with

bacteriostatic activity. One such compound is chlorogenic acid which has been known to have bacteriostatic properties. These results indicate that chlorogenic acid can be used as a marker compound. The result also shows that the bacteriostatic effect of *L. japonica* is the result of many constituents, not only from chlorogenic acid [60].

Chemical marker is the main point of quality control for herbal medicines based on compound-oriented. To meet the objectives as parameters for quality, safety, and efficacy, the selection of compounds used at various stages of developing and manufacturing herbal medicine, from raw materials to finished products, from batch to batch production. Chemical marker has several requirements that must be fulfilled. The chemical structure is known, reference standards are available, and the levels in the sample can be measured using a reliable instrument.

3.2. Phytoequivalence concept

One or a group of marker compounds cannot describe the composition of compounds in phytopharmaceuticals. Multicomponent in phytopharmaceuticals does not constitute the sum of each component' activities in it. Due to various synergistic effects, they form a unity that cannot be separated. In this way, multicomponents in phytopharmaceuticals are considered as "an active ingredient". This active ingredient has been clinically proven used as a standard reference for quality control. This concept is known as "phytoequivalence" developed in Germany to ensure the consistency of phytopharmaceuticals [65].

Multicomponent active ingredients in phytopharmaceuticals can be extracts or fractions of medicinal plants' raw materials. So, this extract or fraction is treated as a single chemical entity in every possible way. However, some difficulties must be faced when applying Good Manufacturing Practices (cGMP) of medicinal products to phytopharmaceuticals. There are many elements in the active ingredients of phytopharmaceuticals, including, active, inactive, unknown compound, and elements that are dietary rather than therapeutic. Another difficulty for this analysis is to provide a standard reference that is always available. The analytical method that meets these conditions is the chromatographic fingerprint. The use of chromatographic fingerprints for phytopharmaceuticals is carried out with a focus on compounds that can be detected by the type of chromatography used with or without knowing the compound' structure [66].

Chromatographic fingerprints are chromatographic patterns of multicomponent of active ingredients that show pharmacologically active chemical components and show chemical characteristics. The chemical components in phytopharmaceutical active ingredients are unknown and many are in low quantities. This makes it very difficult to obtain reliable chromatographic fingerprints representing pharmacologically active components and chemical characteristics. Chromatography (TLC, HPLC, and GC), which has a powerful separation ability, is an appropriate method to meet chemical fingerprinting desires. Furthermore, the application of hyphenated chromatography and spectrometry such as HPLC-DAD, GC-MS, CE-DAD, HPLC-MS, and HPLC-NMR, can provide additional spectral information, which is very helpful in determining the chemical structure of detected compounds. Chemical fingerprint combined with a chemometric approach will provide more accurate analysis results [65].

Several factors will influence in establishing good chromatographic fingerprints. These factors include sample preparation, instrument selection, measurement conditions, and analytical method validation. These factors must sample preparation, the critical points is the method of sample preparation, the critical point is the method of sample

extraction. The selection of instruments must pay attention to the sensitivity, selectivity, ability of the detector, and the measurement conditions. The last thing to do is to validate the method [65].

Fingerprint analysis begins with the matrix construction of peak area data - retention time of the selected peak, known or unknown identity of the compound. The matrix is then analysed to determine the similarity or difference compared to the standard fingerprint. The most important thing in this process is peak detection (integration) and peak selection, which becomes very difficult because of complex samples containing more than 100 peaks. The disadvantage of peak selection is the amount of peak data that will be rejected. For this reason, the most recent chemometric analysis uses all data points collected in the study. Next, chromatographic fingerprints are analysed through comparisons to determine the similarity or dissimilarity of the sample with the reference standard, presented as a correlation coefficient or congruence coefficient. The fingerprint reference standard is derived from the standardized extract of phytopharmaceuticals, which has become a product prototype. Currently, chemometric analysis methods using pattern recognition have been highly developed with various methods such as K-nearest neighbours (KNN), soft independent modeling of class analogy (SIMCA), etc. [65].

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4 Conclusion

The use of chemical fingerprints for quality control purposes only to determines the similarities and/or differences. This objective can be applied from raw material authentication, in-process manufacturing control, and end process control of finished products. But it has not yet considered the complex relationship between chromatographic fingerprints and the efficacy of phytopharmaceuticals. The efficacy of phytopharmaceuticals has the characteristics of a complex mixture of chemical compounds found in herbs. For that reason, the evaluation method of their relationship is not a trivial task. It is not easy to find a suitable method for quality control of phytopharmaceutical. The variability of medicinal plants' chemical content as the raw materials of phytopharmaceuticals is a challenge that must be conquered with the developments of chemical and chemometric analysis methods.

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PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15

PAGE 16

PAGE 17

PAGE 18