DETERMINATION OF CORTICOSTEROIDS IN MALAY TRADITIONAL JAMU USING RP-HPLC-PDA METHOD

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DETERMINATION OF CORTICOSTEROIDS IN MALAY TRADITIONAL JAMU USING RP-HPLC-PDA METHOD

by

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LIST OF SYMBOLS

d_p	Particle diameter
K^0	Specific permeability
ΔP	Pressure
°C	Degree Celsius
A	Absorbance
b	Path length
c	Concentration of solution
F	Flow rate
L	Column length
R	Column radius
r^2	Coefficient of determination
S	Slope
δ	Standard deviation
ε	Molar absorptivity
η	Viscosity
π	Pi

LIST OF ABBREVIATIONS

C. aeruginosa	*Curcuma aeruginosa*
C. domestica	*Curcuma domestica*
C. heyneana	*Curcuma heyneana*
C. longa	*Curcuma longa*
C. xanthorrhizae	*Curcuma xanthorrhizae*
C. zedoaria	*Curcuma zedoaria*
DCA	Drug Control Authority
DXM	Dexamethasone
FDA	US Food and Drug Administration
FTIR	Fourier-transform Infrared spectroscopy
GC	Gas Chromatography
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HCS	Hydrocortisone
HPA	Hypothalamic-pituitary-adrenal
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
LOD	Limit of detection
LOQ	Limit of quantitation
MOH	Ministry of Health
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
NPC	Nasional Poison Centre

NPRA	National Pharmaceutical Regulatory Agency
OTC	Over-the-counter
PDA	Photodiode array
PSL	Prednisolone
QuEChERS	Quick Easy Cheap Effective Rugged Safe
RP	Reversed-phase
RSD	Relative standard deviation
S/N	Signal-to-noise
SLE	Supported Liquid Extraction
SPE	Solid-phase Extraction
SPME	Solid-phase Microextraction
TDLo	Lowest published toxic doses
THM	Traditional Herbal Medicine
US	United State
USM	Universiti Sains Malaysia
USP	US Pharmacopoeia
UV	Ultraviolet
Vis	Visible

PENENTUAN KORTIKOSTEROID DALAM JAMU TRADISIONAL MELAYU MENGGUNAKAN RP-HPLC-PDA

ABSTRAK

Kortikosteroid merupakan bahan campurpalsu yang biasa ditemui dalam ubat herba yang boleh memberikan kesan negatif kepada kesihatan. Terdapat kes-kes yang dilaporkan yang menghandungi kortikosteroid dalam penyediaan Jamu di Malaysia. Tujuan kajian ini adalah untuk menilai sensitiviti dan keberkesanan teknik kromatografi cecair prestasi tinggi (HPLC) dalam pengenalpastian dan pnegukuran jumlah prednisolon, hidrokortison, dan deksametason dalam persediaan *Jamu* konvensional yang dihasilkan di Malaysia. Kajian komprehansif telah dilakukan untuk mengesan kortikosteroid dalam pelbagai sampel dari pasaran Jamu tempatan di Kelantan dan platform seperti Lazada dan Shopee. Sistem RP-HPLC-PDA telah digunakan untuk mengesan dan mengukur analit, iaitu prednisolon, hidrokortison, dan deksametason. Lineariti kalibrasi dalam mengesan steroid terpilih mencapai jumlah r^2 masing-masing adalah 0.999, 0.993, dan 0.951. Salah satu sampel yang dianalisis dikesan mengandungi hidrokortison. Kepekatan hidrokortison dalam sampel dijumpai antara 0.08 mg – 0.10 mg setiap kapsul, dengan nilai RSD 0.19 - 10.17% untuk ujian kerbolehulangan, dan 12.14% untuk ujian kekerapan. LOD untuk prednisolon dan hidrokortison adalah dilaporkan sebagai 1 ppm, manakala ia adalah 0.1 ppm untuk deksametason. Dalam kes LOQ, nilai-nilai yang dilaporkan untuk prednisolon dan hidrokortison adalah 3 ppm, dan 0.4 ppm untuk deksametason. Ketepatan keputusan ditunjukkan oleh nilai pemerolehan semula, iaitu 45%, 65%, dan 75% masing-masing untuk prednisolone, hydrocortisone, dan dexamethasone.

DETERMINATION OF CORTICOSTEROIDS IN MALAY TRADITIONAL JAMU USING RP-HPLC-PDA METHOD

ABSTRACT

Corticosteroid is a common adulterant found in herbal medicines which may bring adverse effects on health. In Malaysia, there are reported cases highlighted the presence of corticosteroids in Jamu preparations. The aim of this study was to evaluate the sensitivity and effectiveness of a high-performance liquid chromatography (HPLC) technique for the identification and quantification of prednisolone, hydrocortisone, and dexamethasone in conventional *Jamu* preparations produced in Malaysia. The study thoroughly examined samples from local *Jamu* markets in Kelantan and online retail platforms like Lazada and Shopee to detect specific corticosteroids. The RP-HPLC-PDA system was utilized to detect and quantify the analytes, namely prednisolone, hydrocortisone, and dexamethasone. The linearity of the calibration curve in detecting the selected steroids achieved an r^2 value of 0.999, 0.993, and 0.951, respectively. One of the analyzed samples was found to contain hydrocortisone with concentration of 0.08 mg – 0.10 mg per capsule (%RSD = 0.19 - 10.17% for repeatability test; 12.14% for reproducibility test). The detection limit (LOD) for prednisolone and hydrocortisone is 1 ppm, while for 0.1 ppm for dexamethasone. For the quantification limit (LOQ), it is 3 ppm for prednisolone and hydrocortisone, and 0.4 ppm for dexamethasone. The accuracy of the results is indicated by the percent recovery values, which are 45%, 65%, and 75% respectively for prednisolone, hydrocortisone, and dexamethasone. The validated method is deemed to be suitable in determining corticosteroids in *Jamu* products.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The term '*Jamu*' is generally refers to traditionally prepared herbal medicines by the Malay Archipelago across the Southeast Asia countries, specifically Malaysia, Indonesia, and Singapore. These Malay remedies are generally phytopharmaceuticals which indicates that they are composed of plant-based constituents, including roots, leaves, bark, and fruits, and have a rich heritage spanning centuries (Tuschinsky, 1995). They have been traditionally prepared in the form of decoction, infusion, or paste, and usually administered either orally or topically (Rashid et al., 2018).

Although there were existing scepticisms among contemporary allopathic medical practitioners towards *Jamu* accounted to insufficient scientific evidence to support the efficacy of Malaysia traditional medicine, *Jamu* is popular among the poorer segments of population due to its low cost. It provides cost-effective healthcare solutions and improve access to healthcare in remotes area where modern medical practices are lack of accessibility (Khatun et al., 2011).

Despite the persistent and deeply rooted role of *Jamu* in traditional Malay health practices, there remains a necessary need for rigorous scientific investigation and regulatory oversight (Lim & Pranata, 2020). This is particularly relevant in light of the commercialization of *Jamu* products, particularly those that deemed to boost immunity and cure ailments. *Jamu,* which derived from the ancient Javanese word 'Jampi', has the meaning of magic potion, is typically ingested to relieve discomfort

and inflammation or treating chronic illness, such as cancers and rheumatism (Mustarichie et al., 2017).

Additionally, the occurrence of adulteration, wherein unauthorized substances such as corticosteroids are added to these remedies without being declared in the product labelling, highlights significant health risks and underscores the vulnerability of consumers (Mustarichie et al., 2017). The reason for the addition of corticosteroids into *Jamu* is due to its pharmacological properties of corticosteroidal activity that have the potential to enhance analgesic effects and anti-inflammatory properties. This action, in turns, leads to a substantial increase in its demand and popularity among the Malay community (Fung & Linn, 2017; Lim & Pranata, 2020; Mustarichie et al., 2017).

In numerous nations, steroids like prednisolone (PSL), hydrocortisone (HCS), and dexamethasone (DXM) are categorized as controlled substances due to their potential adverse health effects. Belonging to the corticosteroid class, the administration of this category has the potential to give rise to undesired outcomes, which encompassing, though not restricted to, the Cushing's syndrome, adrenal insufficiency, peptic ulcer disease, osteoporosis, and immunosuppression, particularly when administered at elevated doses for prolonged durations (Chong et al., 2015; Fung & Linn, 2017).

In regard to this reason, the regulatory framework governing traditional medicine in Malaysia posed a pivotal role in securing public health and consumer confidence. The Malaysia Law, which inclusive of the Food Act 1983, the Poison Act 1952, the Sale of Drugs Act 1952, and the Control of Drugs and Cosmetics

Regulations 1984 provides a legal framework entails a clear prohibition against the adulterant of corticosteroids into traditional medicine.

In April 2016, the Health Ministry issued warning against unregistered Chinese traditional medicine products, namely Dong Mai Tan and Seven Leave Ginseng, due to reported cases of liver failure and other adverse effects. Subsequent tests revealed the presence of dexamethasone, a potent corticosteroid controlled under the Poisons Act 1952 ("Two Traditional Medicines Unsafe to Use," 2016). Similar incidents were also recently recorded in January 2019, investigations uncovered the presence of banned substances, including steroids such as prednisolone and dexamethasone in *Jamu* products that claimed to enhance energy production, thus emphasizing the potential hazards of unregulated herbal remedies (Halina & Fatin, 2019).

These cases demonstrate the importance of adhering to regulatory standards to ensure consumer safety and the need for informed decision-making regarding the consumption of herbal products.

1.2 Problem Statement

Despite the fact that *Jamu* has historically been used as a natural, traditional alternative to modern medicine, there have been concerns that some of its products have been tampered with addition of corticosteroids, which can have adverse effects on health. Corticosteroids in *Jamu*, which have been linked to a number of harmful side effects, inclusive, but not limited to, the liver damage, infertility, cardiovascular disease, Cushing's syndrome, and diminished immunity, are of special concern (Mustarichie et al., 2017; Wai et al., 2007).

The detection of corticosteroids in *Jamu* products, is therefore, an area of notable scientific interest due to their historical significance. Nevertheless, the scientific literature has a considerable lack of research on the employment of RP-HPLC for this purpose. Even though RP-HPLC has been employed to detect certain adulterants such as glibenclamide, there is a shortage of research that specifically focuses on the detection and quantification of corticosteroids (Utami et al., 2019).

This research gap presents a significant challenge in assessing the presence of potentially harmful corticosteroids within *Jamu* products, thereby hindering the comprehensive evaluation of their safety, efficacy, and adherence to regulatory standards. The traditional medicine sector requires the addressing of this gap to enhance consumer protection, ensure the quality of herbal products, and make informed regulatory decisions.

To assure the safety and quality of *Jamu* products, it is necessary to validate effective methods for the detection of corticosteroids in those items. This study aims to address this knowledge gap by assessing HPLC techniques for the detection of corticosteroids which are the prednisolone, hydrocortisone, and dexamethasone in traditional *Jamu* made in Malaysia and to provide a sensitive and accurate approach for their detection.

1.3 Objectives of the Study

1.3.1 General Objectives

The general objective of this study was to analysis of corticosteroids in Malay traditional *Jamu* products using RP-HPLC-PDA method.

1.3.2 Specific Objectives

The specific objectives of this study are as below:

1. To validate the RP-HPLC-PDA method for the detection and quantification of prednisolone, hydrocortisone, and dexamethasone in traditional *Jamu* samples using established method.
2. To optimize the RP-HPLC-PDA conditions for the separation and quantification of prednisolone, hydrocortisone, and dexamethasone in traditional *Jamu* samples.
3. To determine prednisolone, hydrocortisone, and dexamethasone in traditional *Jamu* samples using RP-HPLC-PDA.

1.4 Significance of the Study

The significance of this study is to contribute onto public health, regulatory compliance and quality assurance. By utilizing RP-HPLC-PDA to detect corticosteroids in *Jamu* products, it protects consumers from harmful substances, ensuring the quality of traditional remedies.

Furthermore, this research is valuable in terms of regulatory enforcement. It identifies gaps in adherence and set off valuable measures to prevent the manufacture of adulterated products. This is in line with transparent market principles and benefits both consumers and manufacturers. In medicolegal cases which involves consumption of adulterated *Jamu*, the scientific report of the analysis may become indirect evidence which assist in the medicolegal investigation and litigation.

From the academic perspective, this study addresses the research gap in analytical sciences, thereby holding forensic significance on public health safety. The use of RP-HPLC acts a crucial role, enabling the separation and quantification of not just one, but multiple corticosteroids concurrently, when employing accurate and refined methodologies. The findings will not only enrich the academic discourse on detection of corticosteroids in *Jamu* but also pave the way for future investigations into similar methodologies for assessing other adulterants in other traditional remedies.

In conclusion, this study may positively impact public health, regulatory bodies, and filling of the research gap. By detecting corticosteroids, it ensures public safety and informs health choices. Through its varied implications, this research may foster a safer and healthier society.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

The present literature review delves into a comprehensive study of the knowledge surrounding the identification of corticosteroids in traditional herbal formulations, with a specific emphasis on the Malay traditional medicine commonly referred to as '*Jamu*'. This section is organized in such a way as to provide a thorough comprehension of the analytical methodologies utilized, the consequences of corticosteroid overdose, the regulatory frameworks, and the forensic significance of accurate detection techniques.

2.2 Malay Traditional Herbal Remedies – *Jamu*

The term '*Jamu*', as derived from the Malay and Indonesian language, refers to phytopharmaceuticals that are used in Malay tradition which are prevalent in the Malay community throughout the Malayan archipelago, including Malaysians, Indonesians, and Singaporeans (Tuschinsky, 1995; Zakaria & Zainal, 2017). The medical term 'phytopharmaceuticals' is generally defined as products that are derived from medicinal plants and obtained through a range of processes, such as fresh collection, drying, infusion, powdering, or a combination of them. The formulations of these remedies comprise up to 40 different components, prepared in the form of powder, pills, tonics, paste, or capsules, and are claimed to be able to treat specific health issues and/or promote overall well-being (Tuschinsky, 1995).

Figure 2.1 *Jamu* products sold in local vendors in Malaysia.

Medicinal plants from the *Zingiberaceae* family, known as ginger, including *C. domestica*, *C. longa*, *C. xanthorrhizae*, *C. heyneana*, *C. zedoaria*, and *C. aeruginosa*, are commonly found in *Jamu* formulations (Widyowati & Agil, 2018). Moreover, other plant families such as *Justicia gendarussa* and *Cassia siamea* have also been documented for their use in *Jamu* preparations. In Malaysia, observable *Jamu* products are prescribed from a species of oak known as the Aleppo oak, or its scientific name, *Quercus infectoria*.

2.2.1 Historical Evolution of *Jamu*

Although its origins can be traced back to 18th century, the historical roots of *Jamu* extend as far back as prehistoric times, according to Beers (2001). This fact has been proved by the discovering of Neolithic stones within the famous Borobudur temple located in Jakarta. These stones were reportedly used to grind and carve *kalpataruh* tree (undying trees), which was then pounded into a blend of remedies served for the purpose of women's beauty and healthcare needs.

According to Tuschinsky (1995), there was a notable transition in the production of *Jamu* in the early 20th century. The Indonesian entrepreneurs started producing and selling homemade *Jamu* for profit. This successful small-scale business kickstarted a sequence of developments that shaped the present-day *Jamu* industry. These innovative entrepreneurs and their successors are now among the biggest *Jamu* producers globally, like "*Jamu* Jago," "Air Mancur," and "Nyonya Meneer." These companies follow modern management practices and align with Western pharmaceutical standards. However, it is noteworthy that there are currently very few registered *Jamu* manufacturing companies in Malaysia that meet these Western standards.

Figure 2.2 Famous *Jamu* industry in Indonesia.

2.2.2 Public Insight and Safety Concerns of *Jamu* in Malaysia

In Malaysia, the consuming of *Jamu* products has been driven by attraction arising from their declared health advantages. *Jamu* are claimed to be as a thorough strategy to overall well-being, featuring attributes like anti-inflammatory, anti-diabetic, anti-microbial, and anti-cancer properties (Sumarni et al., 2019; Utami et al., 2019; Wai et al., 2007). The anti-viral properties the producers claimed on *Jamu* products particularly demanded during the COVID-19 pandemic (Lim & Pranata, 2020). Potential of *Jamu* in reducing cholesterol levels, managing hypertension, and generating body heat has also contributed to its widespread popularity (Sumarni et al., 2019).

Among women, *Jamu* is favored for its purported aptitude in relieving menstrual discomfort, managing weight, reducing acnes, and improving skin condition (Sumarni et al., 2019). Furthermore, the conventional practice of postpartum and post-sexual activity vaginal tightening has extended the scope of *Jamu*'s appeal to include intimate health (Theresia et al., 2022). These perceived benefits collectively underscore the multifunctional role that *Jamu* products hold in promoting health and well-being among Malaysians.

As *Jamu* products are generally prepared by individuals with limited scientific knowledge, this has led to a practice of limited ingredient disclosure due to the emphasis on insights acquired from a personal realization or experiences, instead of scientific research. In fact, there are limited evidence or research being disclosed stating that the consuming of *Jamu* is beneficial or harmful (Lim & Pranata, 2020). While this preserves tradition, it raises concerns about potentially harmful substances, adulterants or contaminations.

Rofil et al. (2015) addresses the lack of awareness among middle-aged consumers of the Malay community in Malaysia about the potential consequences of undeclared ingredients in unregistered *Jamu* products, coupled with limited scientific knowledge of food safety, often results in their unknowing consumption of such products. This underscores the importance of enhancing public education and promoting rigorous quality control measures to ensure the safety of traditional remedies.

2.3 Adulterations in Herbal Remedies

The adulteration of herbal medicine is a significant concern that challenges the safety, efficacy, and integrity of these traditional remedies. The act of adulteration can be defined as intentional addition or replacement of foreign substance with similar benefits within a product, with the aim of elevating its potency, and/or reducing its cost (El Beyrouthy & Abi-Rizk, 2013). Adulterants can range from synthetic compounds, closely related species, or pharmaceutical agents. However, unintentional adulterations may also happen sometimes as a result of insufficient knowledge with the original plant, as well as the morphological and aromatic similarities among closely related plant species. Moreover, technical issues and carelessness in the collection of plant samples in the field further aggravates this issue (El Beyrouthy & Abi-Rizk, 2013).

Manufacturers execute the act of adulteration due to various reasons, including increasing mass of product, economic motivations, cost-cutting measures, and increase potency of the product in attempts to capitalize on the market demand for specific health benefits (El Beyrouthy & Abi-Rizk, 2013; Eseller et al., 2020;

Everstine, 2017; Momtaz et al., 2023; Teen Teh & Dykes, 2014). In some cases, adulteration may be driven by a lack of proper quality control measures, inadequate regulatory oversight, and the pressure to produce products quickly to meet consumer demands (Thangaraju et al., 2021).

According to Ariffin et al. (2021), roughly 60,000 THM products were seized in Malaysia due to being unregistered under the appropriate health authorities. An overwhelming majority of about 95% of the total seized items were discovered to have been adulterated. Among the reported adulterants, steroids accounted for approximately 50% of the total proportion. This unethical practice poses notable health risks to consumers, as undisclosed additives can lead to adverse reactions, drug interactions, or even severe health consequences (Gizaw, 2019; Momtaz et al., 2023). Addressing adulteration in herbal medicine products is, therefore, of utmost importance to safeguard public health and ensure that consumers receive safe and genuine, high quality traditional remedies.

2.3.1 Corticosteroids Adulteration in Herbal Remedies

Corticosteroids, a type of steroid adulterant, have consistently been detected in various forms of traditional herbal medicines, covering traditional Chinese medicine (TCM), traditional Indian medicine (Ayurvedic), Thai traditional medicine, Korean herbal medicine, and notably, in the context of *Jamu* products (Bhatia et al., 2003; Mustarichie et al., 2017; Park et al., 2016; Waranee et al., 2021; Xue et al., 2021).

Corticosteroids, including but not limited to prednisolone, hydrocortisone, and dexamethasone, were frequently added into traditional medicine with the intention of stimulating their analgesic and anti-inflammatory effects (Chong et al., 2015). This

strategy was employed in order to attain the purported advantages of relieving pain and managing inflammatory responses as marketed by the vendor (Fung & Linn, 2017).

The adulteration of corticosteroids may give rise to numerous medical complications, thereby resulting in harmful effects on nearly every system within the human body (Tirla et al., 2021; Zoorob & Cender, 1998). Hence, the consequences of corticosteroids must not be neglected. In this work, emphasis will be placed on studying the frequently administered corticosteroids namely prednisolone, hydrocortisone, and dexamethasone.

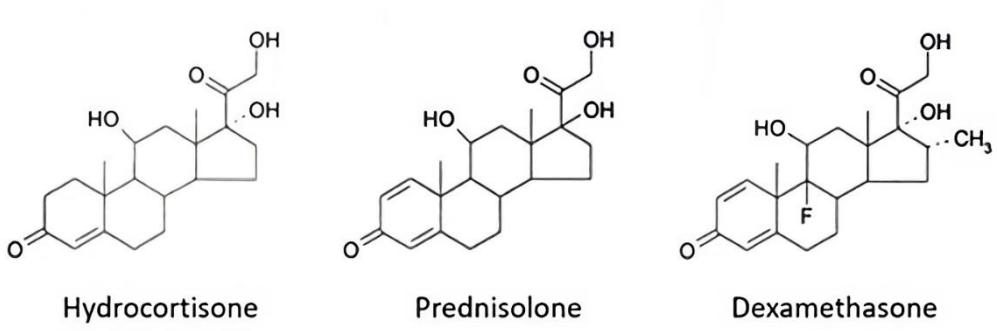

Figure 2.3 Chemical structure of hydrocortisone, prednisolone, and dexamethasone (image source from PubChem).

2.3.2 Prednisolone

The prednisolone (11β,17α,21-trihydroxypregna-1,4-dien-3,20-dione) is a hydrophobic corticosteroid drug, characterized by the double bond formation in the carbon between C_1 and C_2 of the steroid rings, which distinguish it from hydrocortisone. Similarly, to other corticosteroids, prednisolone is used to treat acute management of severe attack, deterioration episodes (Landau & Martinez, 2008). More commonly, prednisolone is administered orally or intravenous to treat autoimmune disorder such as acute asthma, acute gout or rheumatism, and eczema

by acting as an immunosuppressant with the mechanism of increasing of blood glucose level, potassium secretion, and reducing sodium secretion (Vardanyan & Hruby, 2006).

The metabolic process of prednisolone takes place in the liver, wherein it undergoes reversible metabolism to prednisone, followed by several metabolites, and is predominantly excreted through the urine (Matabosch et al., 2015). Acute overdose of prednisolone may be accompanied with symptoms such as disturbance on the gastrointestinal system, and restlessness which may leads to insomnia (Robinson et al., 2016). Chronic overdosing of prednisolone may result in reduced growth, moon face, disturbed hypothalamic-pituitary-adrenal (HPA) axis function, formation of cataracts, and psychological changes arising from hormonal imbalance (Vardanyan & Hruby, 2006). According to Cayman Chemicals Material Safety Data Sheet (MSDS) (2023), the reported human oral TDLo (Toxic Dose Low) in men is 9mg/kg/2W and in women is 14mg/kg/13D.

2.3.3 Hydrocortisone

The hydrocortisone (11β,17α,21-trihydroxypregn-4-en-3,20-dione) is another corticosteroid similarly to prednisolone, but without the double bond in the carbon between C_1 and C_2 of the steroid skeleton. The function and usage of hydrocortisone is similarly as discussed in the section of prednisolone, hydrocortisone also exhibit anti-metabolic activity and lower histamine synthesis (Vardanyan & Hruby, 2006). The potency of hydrocortisone is about 4 to 5 times lesser than prednisolone (Meikle & Tyler, 1977; Zoorob & Cender, 1998). Hydrocortisone is commonly employed in

the treatment of arthritis, renal and adrenal insufficiency, allergic conditions, colitis, as well as collagen and dermatological diseases (Vardanyan & Hruby, 2006).

Hydrocortisone gradually metabolized to cortisone after several prior metabolism process in the liver, and ultimately eliminated through urine (Sarkar et al., 2015). While acute overdose of hydrocortisone is unlikely to happen, chronic overdose of hydrocortisone may include symptoms associated with decreased immunity towards infection, cataract formation, hyperglycemia, hypocalcemia, metabolic acidosis, mood changes, and secondary adrenal insufficiency (Ciriaco et al., 2013).

2.3.4 Dexamethasone

The dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxypregna-1,4-dien-3,20-dione), structurally distinctive to prednisolone and hydrocortisone by the characteristic of an extra fluorine atom at the C_9 carbon of the steroid skeleton. The administration of dexamethasone is indicated in a manner similar to previously mentioned corticosteroids; however, it exhibits significantly stronger potency in its anti-inflammatory and anti-allergic action (Vardanyan & Hruby, 2006). The potency of dexamethasone is about 25 times stronger than hydrocortisone (Meikle & Tyler, 1977; Zoorob & Cender, 1998).

Due to its high potency, the anti-shock properties of this substance are commonly used in cases of circulatory collapse that arise during or after surgical operations, trauma, myocardial infarction, and blood loss (Vardanyan & Hruby, 2006). Furthermore, it is also used in vascular collapse in several severe infections, including typhoid fever, septicemia, meningococcosis, and peritonitis (Vardanyan &

Hruby, 2006). Correspondingly, it is effective in managing severe allergic conditions, such as severe asthma, oedema in the laryngeal, anaphylactic reactions to drugs, and pyrogenic reactions (Vardanyan & Hruby, 2006). In brief, dexamethasone is administered to treat more severe medical conditions in contrast to situations where prednisolone and hydrocortisone are used.

The metabolism of dexamethasone involves enzymatic dehydrogenase activity, leading to its conversion to 11-dehydrodexamethasone in the kidneys, and predominantly eliminated through urine (Diederich et al., 1998). Acute effects resulting from dexamethasone overdose include damage to the kidneys, ureters, and bladder, followed by dehydration due to an increase in urine volume. According to Cayman Chemical's MSDS (2015), the reported TDLo of dexamethasone in women is 4759 µg/kg/18D through multiple routes and 1,800 mg/kg on the skin at a concentration of 10 mM. Chronic overdosing of dexamethasone leads to symptoms similar to those described for its less potent short-acting counterparts, namely hydrocortisone and prednisolone. However, a life-threatening condition known as toxic epidermal necrolysis can be triggered if dexamethasone is combined with Thalidomide, a drug used to treat skin lesions and multiple myeloma (Rajkumar et al., 2000).

Figure 2.4 Patient with toxic epidermal necrolysis (image source from Rajkumar et al., 2020).

2.3.5 Other Corticosteroids

While prednisolone, hydrocortisone, and dexamethasone are the focal points of this study due to their frequent adulteration in traditional herbal products, it is important to acknowledge the presence of other corticosteroids that might also find their way into these formulations. Among them are betamethasone, prednisone, triamcinolone, mometasone, and cortisone. These corticosteroids, though less commonly encountered as adulterants, some have been reported in some cases.

Betamethasone, a synthetic glucocorticoid, is commonly used to manage various inflammatory and autoimmune conditions. While it is not as prevalent as prednisolone, betamethasone has been detected as an adulterant in herbal products, raising concerns about the potential health risks associated with its unregulated use (“Herbal Medicines,” 2016; Mose & Bygum, 2019).

Prednisone, another corticosteroid, is often prescribed to manage conditions such as asthma, allergies, and inflammatory diseases. Its anti-inflammatory and immunosuppressive effects make it effective in controlling immune responses. While prednisone's presence as an adulterant in traditional herbal products might be less widespread, there have been instances of its detection in such formulations (Fung & Linn, 2017).

Triamcinolone, mometasone, and cortisone are additional corticosteroids that merit consideration, even though they have not been commonly identified in herbal medicine adulteration cases. These steroids generally also administered with the purpose of analgesic and anti-inflammatory properties, found to be especially useful on dermatitis conditions (Vardanyan & Hruby, 2006). While the focus of this study revolves around the detection of prednisolone, hydrocortisone, and dexamethasone as potential adulterants, it is crucial to acknowledge the possibility of these less frequently encountered corticosteroids entering herbal formulations.

Although these corticosteroids might not have garnered as much attention in adulteration cases, their potential presence cannot be dismissed outright as there were cases reported the inclusion of these corticosteroids in some illicit skin-lightening cream (Choi & Nisha, 2022).

Given the complex landscape of traditional herbal medicine production and the wide range of compounds that can be incorporated into these products, the inclusion of triamcinolone, mometasone, or cortisone as adulterants is a conceivable scenario. Factors such as supplier variability, cross-contamination during production, or even unintentional mislabelling can contribute to the presence of unexpected substances in herbal products.

2.3.6 Corticosteroid Adulteration of *Jamu* in Malaysia

The cases mentioned below focuses on notable incidences happened in Malaysia's *Jamu* product market that have brought to light the issue of adulteration in traditional herbal medicine. These cases serve to emphasize the hazards associated with unlicensed or contaminated *Jamu* products, specifically those that contain corticosteroids such as prednisolone and dexamethasone. This localized evidence highlights the imperative need for strict regulations, vigilant supervision, and increased awareness of the public surrounding the usage of *Jamu* products in Malaysia.

In July 2014, Datuk Dr. Noor Hisham bin Abdullah, the head director of the Ministry of Health of Malaysia, alerted the public regarding a number of THM (Traditional Herbal Medicine) items that have been found to be unregistered or adulterated, which one example of it consist of a *Jamu* product named Majun Burung Unta, found to be adulterated with dexamethasone. Several reported cases claimed that this particular *Jamu* has been responsible for causing negative effects on health such as Cushing's syndrome, elevated blood glucose level and gain in weight.

In July 2018, a press release was published by the National Pharmaceutical Regulatory Agency (NPRA) stating that a total of 12 products of unregistered traditional *Jamu* products were found to be added with steroids, which include corticosteroids aforementioned (Noor Ain Norman, 2018).

In January 2019, where consumption of *Jamu* purported for energy enhancing led to adverse effects and raised concerns. Investigations uncovered the presence of banned substances, including steroids such as prednisolone and dexamethasone, thus

emphasizing the potential hazards of unregulated herbal remedies (Halina Mohd Noor & Fatin Hafizah Mohd Shahar, 2019).

Figure 2.5 A poster published by the NPRA to announce that certain *Jamu* products was found to adulterated with steroids.

2.4 Regulatory Framework and Enforcement

The examination of regulatory frameworks concerning to traditional herbal medicine is conducted in a critical manner, emphasizing the prohibition of selected substances, such as corticosteroids and the illegalisation of unregistered traditional remedies. Some of the regulations relating to present studied issues inclusive of the Food Act 1983, the Poison Act 1952, the Sale of Drugs Act 1952, and the Control of Drugs and Cosmetics Regulations 1984.

2.4.1 The Food Act 1983

The Food Act 1983 of the Malaysia law plays a crucial role in the regulation of traditional medicine for the purpose of ensuring public health and safety. This extensive legislation gives in the legal framework for the supervision and control of the production, sale, and distribution of diverse food items, which include traditional medicines like *Jamu* products.

This Act classifies traditional medicines as food items and subjects them to rigorous standards and regulations. Manufacturers and vendors are required to accurately label their products in accordance with the Food Act 1983.

In section 15 and section 16 under the Food Act 1983, the law clearly states that labelling that does not comply with required standards, or employing false labelling are considered as committing an offence, which may be convicted to imprisonment or to fine or to both.

2.4.2 The Poison Act 1952

The Poison Act 1952 of the Malaysia law acts as a principal role in the regulation of substances that possess harmful or toxic properties to human health, including corticosteroids. Corticosteroids, being a potent medication, can have profound physiological effects if not administered appropriately or without proper medical supervision.

The Poison Act 1952 classifies corticosteroids as poisons, thereby enforcing strict regulation concerning their distribution, possession, and usage. The law intends to make certain that these substances are only obtainable from registered medical practitioners for therapeutic purposes and not prone to improper use or abuse.

Corticosteroids are listed as Group B poison in the First Schedule poison list of the Poison Act 1952. It falls under Group C poison when preparation of it is intended for external topical use, including nose, eyes, mouth, or throat.

2.4.3 Sale of Drugs Act 1952

The Sale of Drugs Act 1952 in Malaysia represents a crucial component of legislation directly associated with the supervision and management of pharmaceuticals and substances, including steroids. Its primary objective is to govern the sale, distribution, and quality control of drugs, thereby ensuring that the public is provided with access to only safe medications.

The Sale of Drugs Act 1952 establishes rigorous regulations governing their sale and distribution. Specifically, the act described the meaning of adulteration at section 15. Under section 10, the act states that any person who sells adulterated drugs without declaration to the purchasers are considered as committing an offence. They must adhere to precise quality standards and be properly labelled with accurate information. This includes information pertaining to the ingredients, dosage, usage instructions, and potential side effects.

2.4.4 Control of Drugs and Cosmetics Regulations 1984

The Control of Drugs and Cosmetics Regulations 1984 is promulgated under the Sale of Drugs Act 1952, with the objectives to regulates cosmetic and particular drugs, which includes the traditional medicine.

The regulatory framework particularly emphasizes on regulations on registration and licensing of products, including traditional medicines. While being

executed by the Drug Control Authority (DCA), it ensures the safety, quality and efficacy of pharmaceuticals, health and personal care products that are marketed in Malaysia.

It is noteworthy to highlight the regulation of section 7 which stated prohibition against manufacture, sale, and administration of unregistered products. Under its subcategory, section 7A, prohibition is clearly stated against traditional medicines which contain the following substances:

1. Caffeine
2. Nicotinamide
3. Paracetamol
4. Poison within the meaning of the Poisons Act 1952 [Act 366].

It is essential to note that within the scope of this study, corticosteroids fall under Group B poison as classified in the first schedule of poisons list under the Poison Act of 1952. Consequently, the inclusion of corticosteroids such as prednisolone, hydrocortisone, and dexamethasone in traditional medicines, including *Jamu*, constitutes a violation of the law and is considered an offence.

2.4.5 Registration of Traditional Medicine

In Malaysia, all pharmaceutical products, including traditional medicines and health supplements, must undergo a rigorous registration process with the DCA under the Ministry of Health Malaysia before they can be introduced to the market. Each registered product is assigned a distinctive registration number, denoted by the prefix 'MAL', followed by an eight-digit number, and further classified into

categories represented by the letters A, X, T, or N (Manzatul & Mazlan, 2014). For example, a registration number might look like MAL12345678A.

These classification letters signify different categories of registered products which is illustrated in Table 2.1.

Table 2.1 Classification of registered products.

Alphabet	Description
A	This category refers the product as controlled medicines, such as anti-biotics and anti-diabetic drugs that are carefully regulated due to their potent nature. Purchasing of this product required prescription from registered medical practitioner.
X	Products falling under this category are over-the-counter (OTC) medicines that can be purchased without a prescription. This includes common items like fever medications and liniments.
T	Traditional medicines, such as *Jamu* and herbal pills, are categorized under this category. These products must adhere to the use of only natural ingredients and exclude any modern or controlled medicines.
N	Health supplements containing vitamins, minerals, and other nutritional components fall within this category, focusing on enhancing overall well-being.

It is essential to note that the classification of products ensures that they align with their intended use, safety, and regulations, fostering consumer confidence and safeguarding public health. Traditional medicines like *Jamu* are subject to the same rigorous registration standards, ensuring that they meet quality criteria and consist solely of natural ingredients.