EXTRACTION THE DAYAK ONION (*Eleutherine sp*): SCIENTIFIC BASED HERBAL MEDICINE (OHT) PRODUCTION PROTOCOL

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Abstract: Indonesia has the second largest tropical forest and the richest in flora biodiversity in the world. Based on the Basic Health Research 2010, 59.29% of Indonesian had consumed herbal medicine, noted that Indonesian had consumed herb since the VIII century. Dayak onion (Eleutherine sp) widely used as traditional medicine, as a bahimang /wound healing agent. Dayak onion is not reassured as a medical treatment and lacked scientific evidence. Until 2018, thousands of herbs consumed by Indonesian, only 85 ingredients clinically tested. Sixty-four herbs have been pre-clinically tested and designated as Scientific based Herbal Medicines/Obat Herbal Terstandar (OHT) and 21 herbs have been tested clinically and determined as Phytopharmaca by the Badan Pengawasan Obat dan Makanan Republik Indonesia (BPOM-RI). Based on the data, the Dayak onion appointed as a Scientific based Herbal medicine (OHT) based on pre-clinical and phase 0 clinical research. Potential and promising for the treatment of metabolic, degenerative, catastrophic, neoplasmic, genetic and infectious diseases, Dayak onions can be developed into phytopharmaca in the future. The study aim is to make guidelines for the standardized herbal medicines development according to the rules and regulations.

Keywords: Dayak Onion, *Eleutherine sp*, Scientific based Herbal Medicines, Phytopharmaca, Pre Clinic and Clinical Trials phases

INTRODUCTION

Indonesia has the second largest tropical forest and the priceless biodiversity of flora and fauna as natural resources after the Amazon. The plant used wisely. Example, wood is used for homes and household utensils. Medicine used the leaves, flowers, and roots of plants according to the local wisdom population.[1,2] Prescriptions and documents about traditional medicine were found, Proving that Indonesian ancestors used traditional medicine. The Ancient medicine writings on leaves named: Husodo (Javanese), Usada (Bali), Lontarak Pabbura (South Sulawesi), Primbon Jampi Fiber and Serat Racikan Boreb Wulang Dalem. Reliefs in Borobudur temple (750-800 M) and Prambanan temple (850 AD) and recipes of Sekar Pangkur Sri Mangkunagoro IV (1811-1881 AD) discovered in 1994 are evidence of traditional medicine that has long been used in Indonesia.[3] Mesoneolytic fossil evidence found in the form of alu, pipisan and lumping, the alleged use of herbs began before the XVIII century.[1,2] The traditional treatment came from various plants is called herbs.

Jamu or Djamoe comes from the Javanese and interprets as a prayer or medicine to improve health. More than 50% of Indonesian consume jamu as part of the culture and natural wealth.[4] According to the 2010 Basic Health Research Report, 59.29% of Indonesians have drunk herbs.[3] At present, as many as 9,600 species are known to have medicinal properties. The raw materials drug industry only used 200 species (4%). Various empirical evidence and research related to natural ingredient medicine are being explored to find new drugs and tend to increase. The back to nature issue plays a role in enhancing natural material based research.[4]

The Kalimantan islands have tropical forests of approximately 25.5 million hectares and 4.8 million hectares of peat swamp forest. Many treatments used various plants, especially Dayak onion. Empirically Dayak onions have wound healing benefits.[5] Phytochemical analysis proves that Dayak onions contain the active ingredients naphthoquinone, anthraquinone, flavonoids, tannins, and phenols. The active ingredient is known to have anti-inflammatory and anti-oxidant abilities.[6,7] WHO Global Survey 1994, mentioned the lack of data on research, control mechanisms, education, training and expertise.[3] Many studies are carried out under the standards of the Food and Drug Monitoring Agency/ Badan Pengawasan Obat dan Makanan (BPOM). Most physician practitioners still not to apply in daily practice.[1]

BPOM data (2018) states only 64 herbs have succeeded through the preclinical research stage and are designated as Scientific based Herbal Medicines (OHT), for example, Lelap, Diapet. Also, 21 herbs have gone through the clinical research stage and are designated as phytopharmaca by the BPOM, i.e., Nodiar, X-gra.[6]

Many treatments have still not been satisfied yet: metabolic diseases (diabetic, hypertension, gout metabolic disorders), degenerative diseases (Parkinson's disease, Alzheimer's disease), catastrophic diseases due to bad lifestyle (diabetes, obesity), neoplastic disease, genetic diseases, and infections. The empirical evidence and research of Dayak onions (Eleutherine sp) have the potential and prospect of being Scientific based Herbal Medicines (OHT) and quality phytopharmaca. The study must be the correct and standard rule. The study aim is to make guidelines for the Scientific based Herbal Medicines development according to the BPOM rules and regulations so that Dayak onions can be determined as Scientific based Herbal Medicine (OHT) or Phytopharmaca.

SCIENTIFIC BASED HERBAL MEDICINE (OHT) PROTOCOL ACCORDING TO BPOM

Indonesian Natural Medicine (OBA) is derived from a plant, animal, mineral, cider, or mixture ingredients and used for experience based on generation to generation treatment. There are two types of raw materials for natural medicine, namely simplicia and galenic. Simplicia is dried medicinal ingredients, while galenic are ingredients resulting from extraction, fractionation or subfractionation. Natural Medicines (OBA) classified as Empirical based Herbal Medicine; Scientific based Herbal Medicine (OHT) and Fitopharmaca based on the method of manufacture, type of claim and level of evidence of efficacy.[8-10] The three differences of the OBA are evidence of the effectiveness. Empirical-based Herbal Medicine is empirically safe and does not require clinical trials. Scientific based Herbal Medicine (OHT) efficacy has proven through preclinical testing/modifying of clinical trials, general or medium level quality and BPOM registration requirements. Phytopharmaca is proven to be safe through clinical trials, high quality and mandatory registration of BPOM (table 1).[3,9]

Table 1. Classification of Indonesian Natural Medicine. Herb: leaf twigs in a circle. Standardized based herbal medicine (OHT): 3 pairs of leaf fingers in a ring. Phytopharmaca: the shape of the star of the leaf finger in circles.[3,9]

Parameter	Obat Bahan Alam		
	Jamu (*Empirical based herbal medicine)*	Obat Herbal Terstandar (Scientific based herbal medicine)	Fitofarmaka (Clinical based herbal medicine)
Symbol			
Definition	Traditional medicine served traditionally contains all plant material which is the constituent of herbs.	Traditional medicine presented from extracts or extracts of natural ingredients derived from plants, animals, and minerals.	Natural medicine equal to modern medicine because of the standardized manufacturing process.
Safety	Safe	Safe	Safe
Proof of the efficacy	empirical	preclinical testing / modification of clinical trial	clinical trials
Quality	general quality requirements	general or medium level	Mediun or high
BPOM registration	No regristration	Need regristration	Need regristration

Dayak Onion empirically used to revitalize the body, wounds treatment, and antidiabetic. Dayak Onion has several regional names: si marbawang-bawang (North Sumatra), brambang sabrang (Java), bawang-sabrang(Sunda), bawang Dayak (Pontianak), bawang hutan (East Kalimantan), bawang tiwai (Kutai).[6,7,11]

The taxonomy and phytochemicals of the Dayak onions are: Taxonomy. According to the *Angiosperm phylogeny* Group III system (2009): Kingdom: *Plantae*; Class: *Equisetopsida*; Subclass: *Magnoliidae*; Suborder: *Lilianae*; Order: *Asparagales*; Family: *Iridaceae*; Genus: *Eleutherine*; Species: *Eleutherine sp*.[12]

Based on the PubChem Open Chemistry Database, the Structure of Simplified molecular-input line-entry system (SMILES) and the bioactive 3D (3D), the Dayak onion component (Eleutherine sp) consists of triterpenoids (CID 451674), anthraquinone (CID 6780), eleutherinol / eltherinone (CID 15559106), elecanacin (CID 102091822), eleutheroside-a (CID 101855622), eleutherine (CID 10166), isoeleutherine (CID 10445924), eleutherol (CID 120697), eleuthoside-b (CID 95224384), isoeleutherol (CID 10800314)), dihydroeleutherinol (CID 102473740), honconin (CID 110108147), naphthol

(CID 8663) and naphthoquinone (CID 8530).[6,11,13-16]

GOOD CREATION AND CERTIFICATION TRADITIONAL DRUG (CPOTB)

The two steps to discover new drugs are finding and developing (Figure 1). Based on the empirical, it hoped that Dayak onions could become Standardized Herbal Medicines and Phytopharmaca. The Dayak onion has the potential to stop or reverse the effects of the disease. Molecular bioactive compound analysis of Dayak onion has the potential to be new drugs work on specialized organ systems or genetic manipulations.[17-19]

At the development stage, the Dayak onion potential compounds were conducted identification and experimentation. We have gathered information about: absorption, distribution, metabolism and excretion; benefits and working mechanism; the best route of administration; the side effects and toxicity; the results if given two different sexes, races or ethnics; interaction with other drugs and treatment methods; and effectiveness compared to similar drugs.[18,20]

Before marketing, a new drug must go through a developing process, starting concept, developing active substances, manufacturing processes, analyzing and testing of non-clinics, clinical trials. The proofing process of safety, efficacy, and quality of drugs in humans and the data is used for drug registration to BPOM.[18]

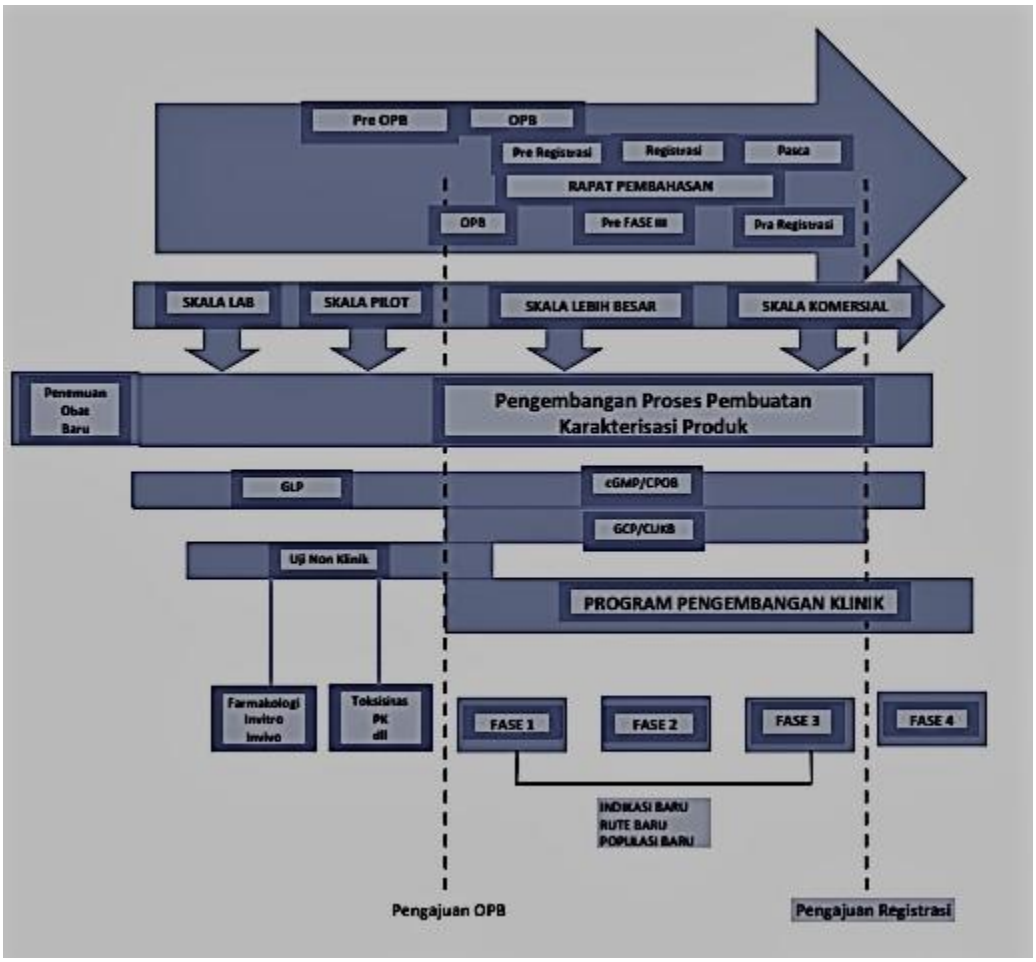

Figure 1. The stages of the New Development Drug (OPB) process according to BPOM. Become Standard Herbal Medicine must meet pre-clinical research. Become a standardized Phytopharmaca must meet clinical research.[18]

Verification and certification are required. Dayak onion needs CPOTB certification to maintain the quality and health requirements; BPOM must establish standard quality assurance consistently. An audit is necessary a technical requirement because OHT and phytopharmaca are used to save lives, or restore or maintain health [21,22]

The next is registration. Registration includes changes of formula varying, methods, industry, production sites, specifications of raw materials and products, containers packaging and markings. The composition is a qualitative and a quantitative arrangement of ingredients efficacious and needs for active ingredients and additional ingredients. Signing is a complete description of the efficacy, safety, and method of use and other information deemed necessary. Markings included the label and brochures included in the package. Containers are for direct contact contents materials, and packaging is for indirect contact substances. Bets are information about the manufacturing cycle related to uniformity and quality.[18]

QUALITY MANAGEMENT SYSTEM (QMS) OF BPOM.

The Quality Management System is a Standard Operating Procedures (SOP) including the format and records of Work Instructions.[23] The problem is Progress of Transportation technology, the international trade lack of restrictions, and the distribution to the community became very rapid. Irrational use of drugs and security priority above prices are a significant problem in society. The effective and efficient Drug and Food Control System (SisPOM) is needed. The purpose of SisPOM is to detect, prevent and supervise medicines to protect public safety, security and health.[23] It should be noted, i.e.: Raw Materials Standardization. Determination aims to identify species and certify the raw material of Dayak onions (*Eleutherine sp*). Some agencies designated for plant determination are UPT Meteria Medika Batu Malang, Indonesian Institute of Sciences (LIPI) Botanical Garden Purwodadi, LIPI Botanical Gardens Bogor, School of Biological Sciences and Technology Institute Technology Bandung.

Harvest. At 2-12 weeks, the development of the leaves and tubers anatomical structure is optimal in diameter and length. The thickness of the mesophyll epidermis, the increase in parenchymal cells and blood vessels are directly proportional to the level of chemical bioactivity. Kuntorini and Nugroho, 2010 recommend harvesting Dayak onions at 12 weeks.[24]

Raw Material Management. The Fresh, simplicia, sweets and powder of Dayak onion bulbs empirically utilized in medicine. Drying by obtained, the water content and active ingredients of simplicia will decrease. Drying must be quick at an optimal temperature. Chemical changes in the content of active compounds can happen at high temperature. The ideal temperature is between 40 - 60^0C for 6 hours. The simplicia moisture content has 10%.[25,26] These techniques are following the CPOTB standard.[18] The harvested, washed, and slowed decay Dayak onion bulbs have stored in the refrigerator. Dayak onion bulbs are chopped 1-2 mm thick, dried in an oven at 50^oC for 6 hours, cooled and packaged in a plastic bag.[26] The right way to produce active drug raw materials (CPBBAOB) aims to control the standard quality.[27,28]

Maceration is a recommended method. The substance tested for its moisture content is mashed by using a blender, and sieved with a B-41 filter to obtain a dry powder. Dry powder weighed as much as 300 g, added 96% ethanol as much as 1000 ml and left for three days. The extract filtered with a filter obtained the 1st filtrate, accommodated in bottles and pulp I plus ethanol 96% 1000 ml again, left for three nights. The extract was filtered with filter paper to obtain the 2nd

filtrate. Using the same technique captures the 3rd filtrate. All the 1st, 2nd, and 3rd filtrates are combine processed, filtered and concentrated. The extract accomplishes concentrated by a Rotary Vacuum evaporator at 400C until extracts are obtained.[29,30] Ethanol is a universal polar solvent, get easily, and often used for extraction. Ethanol is readily soluble in water and has a hydroxide (OH) group so that active substances are more natural to find large quantities.[30]

Ethanol-solvents used for maceration methods. The Fresh Dayak onion bulbs finely blended with ethanol solvent with a 1: 4 (b: v) ratio. The solution was soaked in a sanator for 30 minutes, shaken in the incubator for 2 hours, centrifuged in the Eppendorf, and filtered with Whartman 1 filter paper in the dissolved phase to get the extract solution. Dry for 2 x 24 hours to get dry powder. The dried-extract produced and calculated in the rendemen equation.[31,32)]:

$$Rendemen = \frac{gram\ extract}{gram\ Dayak\ onion} x\ 100\%$$

The dry powder must be simplicia and homogeneous. The dry powder must follow the requirements: Organoleptic, i.e., evaluation on shape, taste, smell, and color. The water content less than 10%, mold number (fungus/yeast) less than 10, zero microbial pathogens, aflatoxin level total (aflatoxin B1, B2, G1 and G2) ≤ 20 µg kg, the aflatoxin B1 ≤ 5 µg/kg (parts per million), Metal/Pb contamination: Pb: ≤ 10 mg kg or mg/L or ppm; Cd: 3 0.3 mg/kg or mg/L or ppm; Ax: ≤ 5 mg/kg or mg/L or ppm; Hg: ≤ 0.5 mg/kg or mg/L or ppm without preservatives, well-closed containers, stored at room temperature, in a dry place and protected from sunlight.[18]

Provisions for equipment include: not causing debris or adverse effects product, weighing equipment, measuring, testing, and regularly record accurately according to appropriate programs and procedures. The filters may not use asbestos materials. Materials for special purposes must not be in direct contact with the processed stuff, i.e., lubricating materials, moisture absorbent materials, and condenser water. Scientific based Herbal Medicines processing equipment must meet the specified requirements, i.e., material extraction equipment, produce a homogeneous mixture, granulation tool or machine for capsule preparations, granule dryers or tools, capsule filling devices or tablets which can supply the combination of ingredients into capsules with uniform weights, tools and primary packaging machines.[33]

Packaging Rules. Scientific based Herbal Medicines and phytopharmaca packaging have strict rules from BPOM. The design of packaging that is not compliant to BPOM and un-registration is illegal.[21] BPOM Packaging Design Rules must include Brands, Illustrations, Efficacy, Registration Number, and Logo. Logo on the upper left must match to the color. There are Manufacturer name, Product composition, Warning/Attention (additional from BPOM), and Net/Contents. BPOM must certificate Traditional medicine product efficacy on the packaging. Packaging includes the method of storage so that the product content is not easily expired, dosage, production number and expiration date, and easy to check the production date. The other things, i.e., consumers filing complaints from dissatisfaction and halal logo.[34]

Before requirements, Employees undergo periodic health evaluation. Packaging and product staff have recovered from injury/disease while work again. Before entering, crews have to hands wash and maintain cleanliness. Only healthy employees can handling, packaging, and finishing raw materials. Reports to superiors if there is a decrease in product quality. Uniforms and protector (masks, gloves) must be adapted. Smoking, eating, and drinking prohibited in the production and storage room.[33]

Pre-clinical research

The Dayak onions pre-clinic test is a biomedical study in vitro on cell culture and in vivo in experimental animals. The aim is to obtain the efficacy/effectiveness, toxicity and pharmacokinetics of the new drug/material to decide whether it is useful to carry out further testing.[18,35,36] In-silico is a computational test to assess the interaction between Dayak onion bioactive ingredients and their targets.[37]

The in-silico study proved that eleutherine and isoeleutherine (*Eleutherine sp*) have anti-inflammatory properties. These compounds can bind to prostaglandin as an inflammatory mediator, via cyclooxygenase enzymes (COX-1 and COX-2). Through hydrogen bonds with Glu353 and Arg394, eleutherol compounds have the potential to inhibit estrogen receptor alpha as the prevention of breast cancer.[16]

In vitro studies, eleutherinol; 1,5-dihydroxy-3-methylanthraquinone compounds; (-) - isoeleutherin; (+) - eleutherin; (-) - hongconin; (+) - dihydroeleutherinol the Dayak onion (*Eleutherine sp*) inhibit nitric oxide in RAW 264.7 lipopolysaccharide-activated in rat macrophages.[4] Dayak onion showed to inhibit NfκB in macrophage cells[5] and bone marrow lipopolysaccharide dendritic cell[15] Dayak onion extract is safe and effective. Three active compounds of eleutherol, eleutherinoside A, and eleuthoside B inhibit the activity of rat intestinal a-glucosidase enzymes that play an important role in intestinal glucose metabolism.[38] Ethanol extract of Dayak onion has good antimicrobial and antifungal activity; its active compound can suppress the growth of six types of gram-positive germs, seven germs of gram-negative bacteria, six species of fungi and two yeasts. The growth of *Aspergillus niger, Rhizopus spp.* and *Penicillium spp.* and the dermatophyte yeast can be inhibited, proving that Dayak onion extract has the potential as an antifungal[39] and antiparasitic.[12] The Dayak onion extract has the antioxidant ability weaker than vitamin C.[24]

In vivo studies prove that the flavonoids and phenolics have high levels of antioxidant ability. These compounds can resonate to phenoxyl radical formations, sinamic acid derivatives, and benzoic acid derivatives. Radical antioxidant compounds of Dayak onion are more stable and harmless to body cells. Dayak onion extract improved the testicular seminiferous tubule function due to cigarette smoke exposure.[40] The ethanol extract of Dayak onion can control hyperglycemic mice[32,41] and repair pancreatic cells.[32] The Dayak onion can improve wound healing through the balance of collagen production.[42]

Phase 0 clinical trials also called first in human (FIH) between preclinical and clinical trials.[40] Phase 0 clinical trials evaluate the side effects of new compounds. The research aims to save costs, time and efficiency in the next experimental phase.[35] Phase 0 clinical trials can be done in a small group of healthy volunteers (10-20 people), to assess pharmacokinetics and pharmacodynamics, to determine the safety, effectiveness, and efficacy of the drug/compound.[17,35,43] Research in Samarinda proves that the dried Dayak onion can reduce cholesterol levels in 12 volunteers.[44]

The Pre-clinical trial is the development of new drugs/compounds to identify of drug targets; development of biological activity analysis (bioassay); drug detection test; establish effective doses and toxic doses; files needed for approval of new drugs. Pre-clinical testing aims to assess the safety, effectiveness, and efficacy of new drugs/methods.[17] Making extracts in research conducted is by the standards set and generally using water, methanol and ethanol solvents classified as polar solvents because of polar components.

The clinical trial involves human subjects and includes the intervention of

test products, to assess clinical effects, pharmacological pharmacodynamics, identify unwanted reactions. Clinical tests observed absorption, distribution, metabolism, and excretion to ensure the safety and effectiveness of new drugs.[18] There is no data on Dayak onion clinical trial research, and cannot be determined as phytopharmaca.

CONCLUSION

Based on the Food and Drug Supervisory Board of the Republic of Indonesia (BPOM) stage determination, the Dayak onion extract can be classified as a Scientific based Herbal Medicines (OHT). Potential and prospective Dayak bawang extracts as phytopharmaca (Clinical based Herbal Medicine) for the treatment need clinical research stages according to BPOM standards.

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