Original Article

JHSMR Journal of Health Science and Medical Research

# Physicochemical Property Determination of Antidiarrheal Remedy of *Ya-Gae-Bid-Na-Ron*

Suhaila Doloh, B.Sc.[1], Siprapha Naknoi, B.Sc.[1], Dujhathai Anekchai, M.Sc.[2], Juraithip Wungsintaweekul, Dr.rer.nat.[1]

[1]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

[2]Pharmaceutical Laboratory Service Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

Received 18 July 2023 ● Revised 21 July 2023 ● Accepted 21 July 2023 ● Published online 26 October 2023

## Abstract:

**Objective:** This study aimed to determine the physicochemical properties of *Ya-Gae-Bid-Na-Ron* (YGBNR); according to the Thai Herbal Pharmacopoeia (THP) guidelines.

**Material and Methods:** Kratom, ginger, Java long pepper, and chilli were collected from different sources, authenticated, and prepared for crude drugs. From this, twelve YGNBR recipes were formulated, and the physicochemical properties of the raw materials and recipes were determined. The thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) fingerprints were investigated, and the microbial limits and heavy metal contamination were determined.

**Results:** YGBNR was prepared by mixing dry powders of kratom: ginger: Java long pepper; chilli in the ratio of 72:5:13:10. The phytochemical properties; including loss on drying, total ash, acid-insoluble ash, ethanol (80% v/v)-soluble extractive, water-soluble extractive, volatile oil, and mitragynine content were: 7.68±0.04%w/w, 5.72±0.06%w/w, 0.26±0.02%w/w, 28.99±0.17%w/w, 21.98±0.21%w/w, 0.40±0.00%v/w, and 0.58±0.28 %w/w, respectively. The microbial limits and heavy metal contaminations were under the THP requirements.

**Conclusion:** The physicochemical properties of YGNBR, an antidiarrheal recipe, was determined according to THP. The specification is essential information for the establishment for the herbal monograph of YGBNR.

This paper was from The 7th Current Drug Development International Conference 2023 & The 1st World Kratom Conference (CDD2023 & WKC2023, August 22-25, 2023).
Contact: Juraithip Wungsintaweekul, Dr.rer.nat.
Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112, Thailand.
E-mail: juraithip.w@psu.ac.th



J Health Sci Med Res .....
doi: 10.31584/jhsmr.20231001
www.jhsmr.org

**Keywords:** antidiarrheal agent, kratom-containing recipe, physicochemical properties, Ya-Gae-Bid-Na-Ron

## Introduction

*Ya-Gae-Bid-Na-Ron* (YGBNR) is a remedy for diarrhea. It is composed of nine leaves of kratom (*Mitragyna speciosa* (Korth.) Havil.), one slice of ginger (*Zingiber officinale* Roscoe) rhizome, one fruit of Java long pepper (*Piper retrofractum* Vahl), and one fruit of chilli (*Capsicum frutescens* L.). YGBNR is one of the thirty remedies that has been officially announced by the Department of Thai Traditional and Alternative Medicine, Ministry of Public Health of Thailand. It was documented in *Tam Ra Ya Kret* No. 259; wherein, it was indicated for the ailment of diarrhea with flatulence, indigestion, nausea, and vomiting[1]. YGBNR contains kratom leaves as a principal ingredient, while ginger, Java long pepper, and chilli are supportive medicines. In Thailand, kratom is used as a traditional medicine; wherein, local people chew 2-3 kratom leaves for refreshment and an energy booster for sun tolerance while working in fields. Furthermore, kratom can be used to treat cough, diarrhea, and pain[2]. On the other hand, a combination of kratom with *Solanum* spp., or *Lagerstroemia speciosa* has reported to exhibit antihyperglycemic activity[3].

Thai Herbal Pharmacopoeia (THP) illustrates the specifications of Thai medicinal plants for quality control[4]. It is a guideline for standardizing Thai herbal medicines, extracts, and formulations existing in Thailand. The methods of analysis for each specification are well described. For multiherbal recipes, the quality requirement is published in the Thai Herbal Preparation Pharmacopeia (THPP)[5]. The plants authentication, identification, purity tests, and determination of active ingredients are reported in the THP and THPP as a monograph. The monographs of ginger, Java long pepper and chilli are described in the THP; however, there is no monograph of kratom. Moreover, THPP has no monograph of YGBNR published as of yet. Due to a standardization of the recipe, the herbal monograph of the YGBNR remedy is required and essential for quality control.

## Material and Methods

### Plant materials

Kratom was collected from the provinces in the southern region of Thailand; from October-November, 2021. The voucher specimens were deposited at the Department of Biology, Faculty of Science, Prince of Songkla University (PSU), Songkhla, Thailand. Ginger rhizomes, Java long pepper fruits, and chilli fruits were purchased from suppliers in Thailand. Table 1 indicates the origin of the plant materials.

Deteriorated samples were picked and discarded. Then, the samples were washed with tap water and air-dried. The kratom leaves, and chilli fruits were dried in a hot-air oven, at 50°C for 24 h. The ginger rhizomes were cross-sliced (0.5 cm wide) and dried in a hot-air oven, at 40°C for three days. The whole fruits of the Java long pepper were dried at 50°C for two days. All plant samples were ground to powder in a Cyclone Mill Twister and sieved (No. 60; particle size 344 µm). The powders were then packed and kept in a dry place until usage.

### Reference standards and reagents

Mitragynine was purchased from Chromadex (California, USA). Capsaicin, gingerol, and the piperine were purchased from ChemFaces Biochemical Co., Ltd (Wuhan, China). Speciogynine, paynantheine, and mitraciliatine were kindly provided from Dr. Niwat Keawpradub, PSU, Thailand. All solvents and chemicals used were of analytical grade.

### Determination of drying index

Thirty pieces of each plant were picked randomly. The fresh weight was weighed, then after drying (under the conditions mentioned above), the remaining weight was recorded. The drying index was calculated as a ratio of the fresh weight and the dry weight.

### Preparation of YGBNR

The formulation of YGBNR was prepared by mixing of 72 g of kratom, 5 g of ginger, 13 g of Java long pepper, and 10 g of chilli in a plastic bag to homogeneity. Twelve formulations of YGBNRs were derived from the mixing of plant materials, which originated from different sources of collection (Table 1).

### Determination of physicochemical properties

The physicochemical characteristics of kratom, ginger, Java long pepper, chilli, and YGBNR were determined using the methods described in THP[4].

### Moisture content

The moisture content was determined using the thermogravimetric method. Two grams of the sample was accurately weighed in a tared-weighing bottle. The sample was put in a hot-air oven, and dried at 105°C for 1 h. After being cooled to room temperature in a desiccator, the sample was weighed. The drying procedure was repeated under the same conditions, until two consecutive weighings did not differ by more than 1 mg[6]. The percent of loss on drying was calculated and expressed in % w/w.

### Water content

The water content was determined using the azeotropic distillation method. The first distillation was performed by distilling 200 milliliter (mL) of toluene, and approximately 2 mL of water for 2 h. It was then allowed to cool to room temperature; upon which the first volume (N1) was taken. Then, fifty grams of the sample were accurately weighed and put into the same flask. This was then continued until the distillation was complete. Afterwards, the second volume was read (N2). The water content in % w/v was calculated as follows:

### Total ash

Two grams of the sample were accurately weighed in a tared crucible, which were then incinerated in a muffle furnace at 450°C until free of carbon. After cooling to room temperature in a desiccator, the crucible containing the ash was weighed. The ignition procedure was repeated under

**Table 1** Formulations of *Ya-Gae-Bid-Na-Ron* (YGBNR) recipes

| Formula No. | Kratom | | | | Ginger | | | Java long pepper | Chilli |
|---|---|---|---|---|---|---|---|---|---|
| | Surat Thani | Nakhon Si Thammarat | Songkhla | Chumphon | Phitsanulok | Phetchabun | Uttaradit | Kancha naburi | Songkhla |
| 1 | • | | | | • | | | • | • |
| 2 | • | | | | | • | | • | • |
| 3 | • | | | | | | • | • | • |
| 4 | | • | | | • | | | • | • |
| 5 | | • | | | | • | | • | • |
| 6 | | • | | | | | • | • | • |
| 7 | | | • | | • | | | • | • |
| 8 | | | • | | | • | | • | • |
| 9 | | | • | | | | • | • | • |
| 10 | | | | • | • | | | • | • |
| 11 | | | | • | | • | | • | • |
| 12 | | | | • | | | • | • | • |

Bullets (•) designate the presence of specified herbs in the recipe. Names of provinces of Thailand under the plant names represent the different sources of collections.

the same conditions, until two consecutive weighings did not differ by more than 1 mg[6]. The percentage of total ash was calculated and expressed in % w/w.

### Acid-insoluble ash

The total ash was boiled in 25 mL of diluted hydrochloric acid (10% v/v) for 5 min. The insoluble matter was collected on ashless filter paper (Whatman No. 42), and the residue was washed with hot water, until the pH of the filtrate was neutral. The acid-insoluble residue was ignited in a muffle furnace at 500°C. After cooling to room temperature in a desiccator, the crucible containing the ash was weighed. The ignition was repeated under the same conditions until two consecutive weighings did not differ by more than 1 mg[6]. The percentage of acid-insoluble ash was calculated and expressed in % w/w.

### Extractive values

In order to determine a water-soluble extractive value, five grams of the sample were accurately weighed and macerated in 100 mL of chloroform water in a closed flask for 24 h. The flask was frequently shaken for 6 h and then left standing for 18 h. After filtering, 20 mL of filtrate was transferred to a tared evaporating dish and dried at 105°C. The residue was dried under the same conditions until a constant weight was achieved. The percentage of water-extractive value was calculated and expressed in % w/w.

As for the ethanol-soluble extractive value, the method was similar as the water-soluble extractive; however, the water was replaced with ethanol; except for the kratom, which used 80% v/v ethanol.

### Volatile oil content

For the ginger and YGBNR, fifty grams were accurately weighed in a round-bottomed flask, and 200 mL of water was then added. For the Java long pepper, twenty-five grams was weighed, and then 250 mL of water was added. The distillation was set and the samples were distilled for 5 h. The percentage of volatile oil content was calculated and expressed in % v/w.

### Preparation of the extracts

Ten grams of YGBNR were macerated with 80% v/v ethanol, by being shaken for 8 h, and further macerated at room temperature for 16 h. The filtrates were collected, evaporated, and kept in the freezer until used.

### Determination of mitragynine content

The mitragynine content in dry extracts of kratom leaves and YGBNR was determined using the high-performance liquid chromatography (HPLC) method, as previously described[7], with modification. The solution of the extract was prepared in methanol in the concentration of 1 mg/mL. One milliliter (mL) of the sample was filtered through a nylon membrane before HPLC analysis. The HPLC system consisted of a Shimadzu Prominence i (Shimadzu, Kyoto, Japan), a reverse-phase C18 column (Vertisep™ UPS; 250 x 4.6 mm, 5 µm) (Vertical, Bangkok, Thailand), and a mobile phase of 35:65 (20 mM $NH_4OAc$; acetonitrile). The Photodiode array was set at 220 nm, and the flow rate was 1 mL/min. The amount of mitragynine was expressed as a % w/w of dry extract.

### Thin-layer chromatography (TLC) fingerprint

The fingerprint of TLC was established using normal-phase silica gel 60 GF254 (Merck, Darmstadt, Germany). The mobile phases were 1) n-hexane: ethyl acetate: triethylamine; 1:1:0.15; 2) n-hexane: ethyl acetate; 7:3; 3) toluene: ethyl acetate; 3:2[4]. The markers were prepared in methanol at a concentration of 1 mg/mL for the mitragynine and capsaicin, and at a concentration of 2 mg/mL for the piperine and gingerol. The test sample of YGBNR was prepared in methanol at the concentration of 10 mg/mL. Five microliters of the markers and the sample were applied on a silica gel plate, using a microsyringe and Linomat 5 (CAMAG®). The TLC plate was run in the mobile phase for 8 cm, then the plate

was removed and dried. The pictures were captured under white light, ultraviolet at 254, 366 nm, using CAMAG® TLC Visualizer 3 and Vision CATS software (Muttenz, Switzerland).

### HPLC fingerprint

The fingerprint of HPLC was established using a qualitative HPLC column, via a modified method[8]. The reference markers; including mitragynine, paynantheine, speciogynine and mitraciliatine, were prepared at the concentration of 0.1 mg/mL. The test sample was prepared by dissolving the extract in methanol at a concentration of 1 mg/mL. The HPLC system was equipped with Shimadzu Prominence i and the column was a reverse phase (C18), 4.6 x 250 mm, 2.6 µm (Kinetex® EVO, Phenomenex). The column was eluted with a gradient elution of solution A: 0.1% v/v formic acid in water and solution B: acetonitrile within 45 min: flow rate was 0.5 mL/min, and the injection volume was 10 µL. The UV detector was set at 240 nm. The temperature of the column was set at 25°C. The retention times of mitragynine, paynantheine, speciogynine, and mitraciliatine were compared.

### Detections of microbial limits and heavy metal contaminations

Formulas 2 and 12 were selected and determined for microbial limits and heavy metal contaminations. The samples were submitted to the Office of Scientific Instrument and Testing (OSIT), PSU, Thailand.

### Statistical analysis

The data were expressed as mean±S.D. The difference between groups was analyzed using One-Way ANOVA; using Statistical Package for the Social Science (SPSS software V26).

## Results

YGBNR is recommended to be used as a fresh herb. It comprises of nine leaves of kratom, one piece of ginger, one fruit of Java long pepper, and one fruit of chili. This present study converted the formula from fresh herbs to dry powders. The average fresh weights were found to be 2.01±0.14 g per kratom leaf, 4.16±0.51 g per one piece of ginger, 3.39±0.30 g per one fruit of Java long pepper, and 3.89±0.69 g per one fruit of chilli. Thus, the estimation of the YGBNR formula in fresh herbs should contain 18.10 g of kratom leaves, 4.16 g of ginger, 3.39 g of Java long pepper, and 3.89 g of chilli. The results of the average drying indices were 3.33±1.23 for kratom leaves, 12.44±4.37 for ginger, 3.53±0.55 for Java long pepper, and 4.77±1.30 for chilli. Therefore, the proportions of kratom: ginger: Java long pepper: and chilli in dry weight were 5.44:0.33:0.96:0.81. On the other hand, YGBNR dry powder contained 72% kratom, 5% ginger, 13% Java long pepper and 10% chilli.

The collection of kratom was from four locations in southern Thailand; including Surat Thani, Nakhon Si Thammarat, Songkhla and Chumphon provinces. Ginger was supplied from Phetchabun, Phitsanulok, and Uttaradit provinces. Java long pepper was bought from Kanchanaburi province, and chilli was from Songkhla province (Table 1). The specifications of ginger, Java long pepper, and chilli were determined according the THP guidelines. Table 2 summarizes the specifications of all crude drugs. The results indicate the quality of the raw materials met the requirements, and could further be formulated for YGBNR.

According to the guidelines of the establishment of herbal monograph of the THP, the physico-chemical properties should be analyzed from at least 12 samples. Therefore, twelve YGBNRs were formulated by mixing the dry powders from different sources; as shown in Table 1. Twelve recipes of YGBNRs were investigated for their physico-chemical properties; as shown in Table 3.

Consideration of the TLC fingerprints, the formula of YGBNR reflected the presence of kratom extract rather than other herbs. As shown in Figure 1, the markers; such as gingerol, piperine, and capsaicin in the formula extracts could not be seen (see lower panel). This evidence was in

agreement with the HPLC fingerprints in Figure 2. Under separation conditions and elution profiles, no difference in the HPLC fingerprints of kratom and YGBNR could be found in the chromatograms.

Heavy metal contaminations in the YGBNRs were determined from formulas No. 2 and 12. These were chosen based on the low and high values of total ash, and the acid-insoluble ash of kratom (Table 2). Formula No. 2 contained kratom from Surat Thani and No. 12 contained kratom from Chumphon. The results in Table 4 indicates that YGBNRs were contaminated with heavy metals; however, this was below the recommended limits. Both formulas were determined for microbial contamination. The results summarize that *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Pseudomonas aeruginosa* were absent in YGBNRs. The total plate count, as well as yeast and molds were found; however, these were below the recommended limits.

**Table 2** Specifications of herbs used in this study, based on Thai Herbal Pharmacopoeia guidelines

| Topic | Origin* | | | | Specification |
|---|---|---|---|---|---|
| | Surat Thani | Nakhon Si Thammarat | Songkhla | Chumphon | |
| Kratom (Mitragyna speciosa (Korth.) Havil. | | | | | MHM |
| ● Loss on drying (%w/w) | 3.36±0.05 | 4.59±0.12 | 3.45±0.05 | 4.89±0.06 | ≤10% w/w |
| ● Total ash (%w/w) | 4.32±0.02 | 4.93±0.02 | 5.63±0.02 | 6.15±0.03 | ≤7% v/w |
| ● Acid-insoluble ash (%w/w) | 0.17±0.02 | 0.15±0.03 | 0.24±0.01 | 0.42±0.02 | ≤1% v/w |
| ● Water-soluble extractive (%w/w) | 19.87±0.10 | 19.81±0.31 | 20.023±0.18 | 21.62±0.40 | ≥16% w/w |
| ● Ethanol (80% v/v)-soluble extractive (w/w) | 30.46±0.13 | 29.21±0.25 | 28.34±0.01 | 28.58±0.09 | - |
| ● Ethanol-soluble extractive (w/w) | | | | | ≥ 8% w/w |
| Ginger (Zingiber officinale Roscoe) | Phetchabun | Phitsanulok | Uttaradit | | THP |
| ● Water (%v/w) | 10.53±0.23 | 10.27±0.61 | 10.40±0.69 | | ≤11% v/w |
| ● Total ash (%w/w) | 2.97±0.01 | 4.26±0.03 | 4.49±0.04 | | ≤10% w/w |
| ● Acid-insoluble ash (%w/w) | 0.14±0.02 | 0.79±0.04 | 0.83±0.09 | | ≤1% w/w |
| ● Water-soluble extractive (%w/w) | 27.61±0.18 | 28.41±0.17 | 25.59±0.31 | | ≥13% w/w |
| ● Ethanol-soluble extractive (w/w) | 22.60±0.47 | 23.77±0.12 | 27.76±0.40 | | ≥5% w/w |
| ● Volatile oil (%v/w) | 1.20±0.00 | 1.20±0.00 | 1.20±0.00 | | ≥0.8% v/w |
| Java long pepper (Piper retrofractum Vahl) | Kanchanaburi | | | | THP |
| ● Water (%v/w) | 11.20±0.00 | | | | ≤13% v/w |
| ● Total ash (%w/w) | 6.58±0.09 | | | | ≤7.5% w/w |
| ● Acid-insoluble ash (%w/w) | 0.32±0.04 | | | | ≤0.4% w/w |
| ● Ethanol-soluble extractive (w/w) | 15.73±0.13 | | | | ≥10% w/w |
| ● Volatile oil (%v/w) | 1.60±0.00 | | | | ≥1% v/w |
| Chilli (Capsicum frutescens L.) | Songkhla | | | | THP |
| ● Loss on drying (%w/w) | 7.73±0.08 | | | | ≤12% w/w |
| ● Total ash (%w/w) | 6.79±0.13 | | | | ≤10% w/w |
| ● Acid-insoluble ash (%w/w) | 0.08±0.00 | | | | ≤1% w/w |

*Origin indicates provinces of Thailand, where the plant materials were supplied.

MHM=Malaysian Herbal Monograph, THP=Thai Herbal Pharmacopoeia

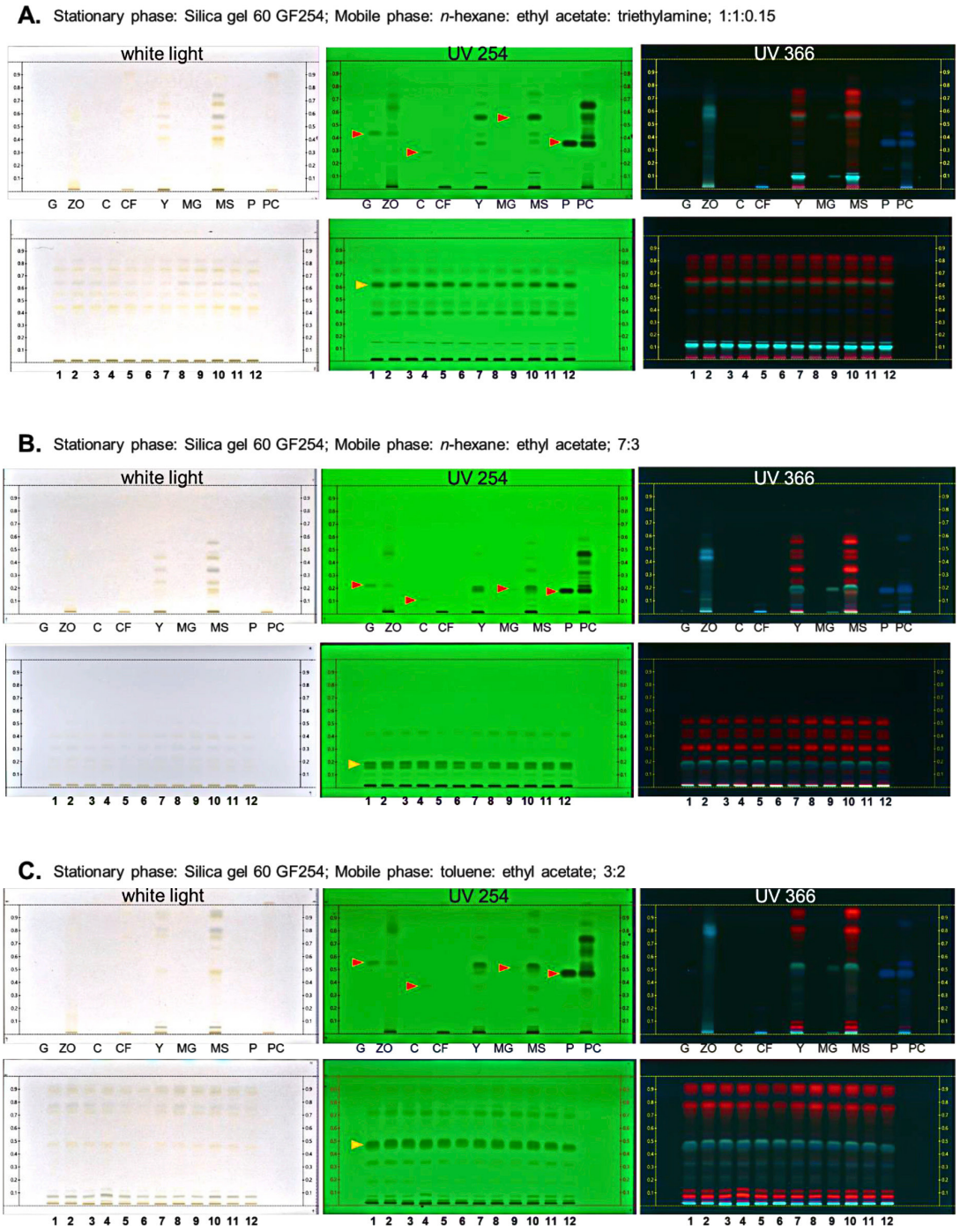



**Figure 1** TLC fingerprints of the YGBNRs, with the markers under different mobile phases. Bands were visualized under white light and ultraviolet light (at 254, 366 nm). Upper panel: markers and the extracts; Lower panel: YGBNRs (formula No. 1–12). Abbreviations: G: gingerol; ZO: ginger extract; C: capsaicin; CF: chilli extract; Y: YGBNR extract No.1; MG: mitragynine; MS: kratom extract; P: piperine; PC: Java long pepper extract. Red arrows indicate the location of markers. Yellow arrows indicate the position of mitragynine

**Table 3** The physicochemical properties of the YGBNR

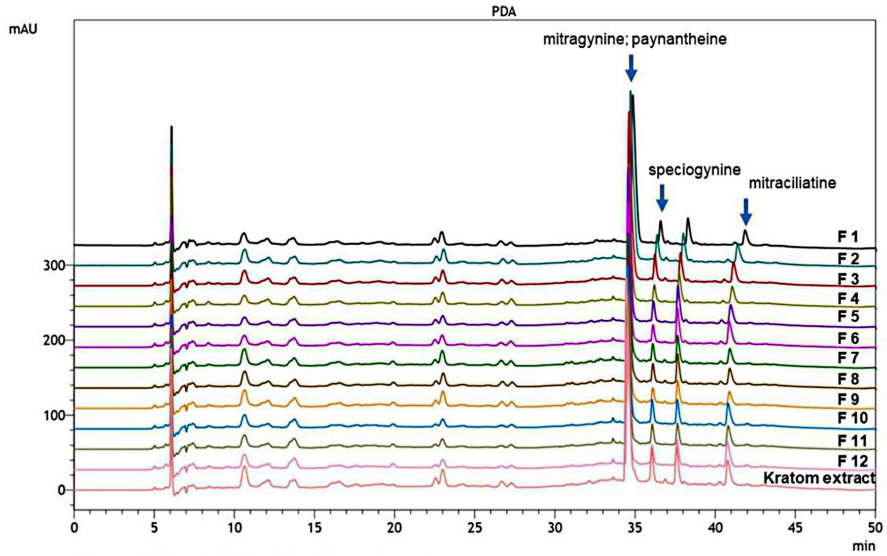
| Formula No. | Loss on drying (%w/w) | Total ash (%w/w) | Acid-insoluble ash (%w/w) | Ethanol (80%v/v) -soluble extractive (%w/w) | Water-soluble extractive (%w/w) | Volatile oil (%v/w) | Mitragynine content (%w/w dry extract) |
|---|---|---|---|---|---|---|---|
| 1 | $7.30^{b}$±0.04 | $4.76^{a}$±0.02 | $0.20^{ab}$±0.02 | $30.35^{bc}$±0.10 | $21.06^{f}$±0.25 | $0.40^{a}$±0.00 | $5.82^{abc}$±0.11 |
| 2 | $7.45^{bc}$±0.45 | $4.89^{a}$±0.04 | $0.25^{bc}$±0.01 | $30.53^{b}$±0.34 | $19.85^{g}$±0.45 | $0.40^{a}$±0.00 | $6.32^{ab}$±0.28 |
| 3 | $7.52^{cd}$±0.05 | $4.97^{ab}$±0.04 | $0.14^{a}$±0.02 | $30.48^{bc}$±0.29 | $21.19^{f}$±0.17 | $0.40^{a}$±0.00 | $5.63^{bcd}$±0.59 |
| 4 | $7.68^{de}$±0.05 | $5.25^{bc}$±0.02 | $0.26^{bcd}$±0.01 | $31.75^{a}$±0.04 | $21.87^{def}$±0.14 | $0.40^{a}$±0.00 | $4.36^{e}$±0.16 |
| 5 | $7.70^{e}$±0.02 | $5.28^{c}$±0.04 | $0.16^{ab}$±0.01 | $30.54^{b}$±0.16 | $22.60^{bcd}$±0.05 | $0.40^{a}$±0.00 | $4.38^{e}$±0.26 |
| 6 | $7.58^{cde}$±0.07 | $5.83^{d}$±0.01 | $0.20^{ab}$±0.07 | $29.21^{de}$±0.07 | $22.49^{cde}$±0.37 | $0.40^{a}$±0.00 | $4.30^{e}$±0.42 |
| 7 | $7.09^{a}$±0.04 | $6.15^{e}$±0.02 | $0.25^{bc}$±0.02 | $29.75^{bcd}$±0.28 | $21.58^{ef}$±0.19 | $0.40^{a}$±0.00 | $3.50^{e}$±0.31 |
| 8 | $7.48^{c}$±0.02 | $5.88^{de}$±0.09 | $0.35^{cd}$±0.01 | $29.65^{cd}$±0.22 | $21.46^{f}$±0.03 | $0.40^{a}$±0.00 | $3.63^{e}$±0.19 |
| 9 | $7.68^{de}$±0.01 | $5.95^{de}$±0.14 | $0.35^{cd}$±0.01 | $29.68^{cd}$±0.32 | $21.39^{f}$±0.18 | $0.40^{a}$±0.00 | $3.48^{e}$±0.31 |
| 10 | $8.20^{f}$±0.04 | $6.53^{f}$±0.08 | $0.37^{d}$±0.04 | $29.06^{de}$±0.06 | $23.70^{a}$±0.08 | $0.40^{a}$±0.00 | $4.24^{e}$±0.13 |
| 11 | $8.20^{f}$±0.02 | $6.56^{f}$±0.07 | $0.31^{cd}$±0.01 | $28.90^{de}$±0.03 | $23.57^{ab}$±0.39 | $0.40^{a}$±0.00 | $4.65^{cde}$±0.26 |
| 12 | $8.23^{f}$±0.05 | $6.64^{f}$±0.13 | $0.26^{bcd}$±0.04 | $28.60^{e}$±0.16 | $22.98^{abc}$±0.20 | $0.40^{a}$±0.00 | $4.61^{de}$±0.30 |
| average± | 7.68±0.04 | 5.72 ± 0.06 | 0.26±0.02 | 29.88±0.17 | 21.98±0.21 | 0.40±0.00 | 4.58±0.28 |
| (α=0.05) | 0.60 | 1.11 | 0.12 | 1.45 | 1.85 | 0.00 | 1.50 |
| Not more than: (1.64S.D.) | 8.28 | 6.84 | 0.38 | | | | |
| Not less than: (1.64 S.D.) | | | | 28.44 | 20.13 | 0.40 | 3.08 |
| Fail | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Not more than (95%CI) | 7.88 | 6.11 | 0.30 | | | | |
| Not less than (95%CI) | | | | 29.39 | 21.34 | 0.40 | 4.06 |
| Fail | 3 | 4 | 4 | 4 | 3 | 4 | 3 |

Statistical analysis (One-way ANOVA; a Scheffe test) was performed. Means with the same letter are not significantly different; a p-value<0.05 is considered significant.
YGNBR=Ya-Gae-Bid-Na-Ron

**Table 4** Detections of heavy metal contaminations and microbial limits in the YGBNR formulas 2 and 12

| Heavy metal contamination | Formula 2 | Formula 12 | THP Requirements |
|---|---|---|---|
| Arsenic | 0.41 | 0.44 | <4 ppm |
| Lead | 1.51 | 0.08 | <10 ppm |
| Cadmium | 0.04 | 0.04 | <0.3 ppm |
| Mercury | 0.07 | 0.07 | <0.5 ppm |
| Microbial limits | | | |
| Total plate count | $5.6x10^{5}$ CFU/g | $4.7x10^{5}$ CFU/g | $<5x10^{5}$ CFU/g |
| Yeast and molds | $2.8x10^{3}$ CFU/g | $3.9x10^{3}$ CFU/g | $<5x10^{4}$ CFU/g |
| *Escherichia coli* | Not detected | Not detected | Absence in 1 g |
| *Staphylococcus aureus* | Not detected | Not detected | Absence in 1 g |
| *Salmonella* spp. | Not detected | Not detected | Absence in 25 g |
| *Pseudomonas aeruginosa* | Not detected | Not detected | Absence in 1 g |

YGNBR=Ya-Gae-Bid-Na-Ron, CFU=colony forming unit, THP=Thai herbal pharmacopoeia




HPLC=high-performance liquid chromatography, YGNBR=Ya-Gae-Bid-Na-Ron

**Figure 2** Alignment of the HPLC fingerprints of extracts of YGBNRs (formula No. 1-12) and kratom extract

## Discussion

The YGBNR recipe contains four medicinal plants; including kratom, ginger, Java long pepper, and chilli. It is one of the official recipes announced by the Department of Thai Traditional and Alternative Medicine, the Ministry of Public Health of Thailand, which is prescribed for the ailment of diarrhea. This study aimed to establish the specifications of YGBNR, in order to establish standardization for the recipe. The plant materials were analyzed according to the methods that are described in the THP. The results revealed that ginger, Java long pepper and chilli qualified when compared to THP requirements. In the case of kratom, this was compared with the specification values of the Malaysian Herbal Monograph (MHM)[9]. Based on the MHM, kratom used in the study met the requirement. However, it must be noted that, instead of an ethanol-soluble extractive, this study used 80% v/v ethanol instead of 95% ethanol.

The YGBNR contained kratom as a principal component, which accounted for 72% of the content in the recipe; followed by: 13% Java long pepper, 10% chilli, and 5% ginger. To establish the specification of YGBNR, twelve recipes were determined for their physicochemical properties. As shown in Table 3, the average values of specifications are stated. The upper and lower limits of the values in each topic were determined. By setting a z-score of 0.05, the maximum values of loss via drying, total ash, acid-insoluble ash, the minimum values of soluble-extractive values, volatile oil content, and mitragynine content are suggested; as shown in Table 3. The outliers were determined at a 95% confidence interval among the 12 formulates of YGBNRs.

The TLC and HPLC fingerprints illustrated that the chemical characteristics of YGBNR was dominantly from kratom. Moreover, the kratom leaf accumulates mitragynine as a major component[9], unlike gingerol in ginger, piperine in Java long pepper, and capsaicin in chilli: these are present in low amounts in the crude drug. It must also be noted that this study used methanol as an extracted solvent; therefore, piperine when dissolved well in chloroform may disappear. Previously, the variation of mitragynine content has been reported to depend upon seasonal and geographical origin[10]. Herein, the mitragynine content in YGBNRs, obtained from

four sources, varied from 3-6% (w/w). Kratom leaves collected from Chumphon and Surat Thani contained a significant mitragynine content, and were responsible for the quality of YGBNR formulas No.1-3 and 9-12, accordingly.

Kratom extract exhibited an antidiarrheal effect, and proposed to act via the opioid pathway[11]. Kratom extract also displayed a gastroprotection[12]. This evidence supports the use of YGBNR for the treatment of diarrhea. The ingredients; such as shogaol and gingerol in ginger have been reported to possess antiemetic and gastroprotective effects[13]. Anti-inflammations of Java long pepper, ginger, and chilli have also been reported[14-16]. Concert action of YGBNR would support the indicative for diarrhea, nausea, and vomiting. Further investigation in vivo and clinical trials would be essential to confirm the traditional use of YGBNR. Therefore, recipe specifications are required to control the quality; as shown in this present study.

## Conclusion

The specifications of YGBNR were determined. This is the first report on the specification of a traditional herbal medicine that contains kratom as a principal component. It is required for the standardization and essential for herbal monograph.

## Acknowledgement

This research was supported by the National Science, Research and Innovation Fund (NSRF) and the Prince of Songkla University (Grant No. PHA6505145M).

## Conflict of interest

The authors declare no conflict of interest.

## References

1. Office for the Management of Cannabis and Kratom in Thai Traditional Medicine [homepage on the Internet]. Bangkok: Department of Thai Traditional and Alternative Medicine; [cited 2023 May 31]. Available from https://ockt.dtam.moph.go.th/
2. Saingam D, Assanangkornchai S, Geater AF, Balthip Q. Pattern and consequences of krathom *(Mitragyna speciosa* Korth.*)* use among male villagers in southern Thailand*:* a qualitative study. Int J Drug Policy 2013;24:351-8.
3. Nakaphan T, Teerachaisakul M, Puttum S, Sompimai K, Nootim P. Traditional use of kratom (*Mitragyna speciosa* Korth.) among folk healers in Southern Thailand. J Thai Trad Alt Med 2016;14*:* 274-84.
4. Thai Herbal Pharmacopoeia [Homepage on the Internet]. Nonthaburi: Bureau of Drug and Narcotic, department of medical sciences, ministry of public health; [cited 2022 October 12]. http://bdn.go.th/thp/
5. Department of Thai Traditional and Alternative Medicine, Ministry of Public Health Thai Herbal Preparation Pharmacopoeia 2nd ed. Bangkok: Siam Charoen Panich (Bangkok); 2020;p.1-241.
6. United States Pharmacopoeia (USP 32). General notices and requirements. Rockville (MD): The United States Pharmacopeial Convention; 2008.
7. Limsuwanchote S, Wungsintaweekul J, Keawpradub N, Putalun W, Morimoto S, Tanaka H. Development of indirect competitive ELISA for quantification of mitragynine in kratom (*Mitragyna speciosa* (Roxb.) Korth. Forensic Sci Int 2014;244:70-7.
8. Cai B, Ong SP, Liu X. High performance liquid chromatography fingerprinting technology of the commonly-used traditional Chinese medicine herbs. Beijing China*:* Chemical Industry Press 2012;1-73.
9. Malaysian Herbal Monograph Committee. Malaysian Herbal Monograph: *Mitragyna speciosa* (Korth.) Havil. (leaves) 2015; 428-36.
10. Sengnon N, Vonghirundecha P, Chaichan W, Juengwatanatrakul T, Onthong J, Kitprasong P, et al. Seasonal and geographic variation in alkaloid content of kratom (*Mitragyna speciosa* (Korth.) Havil.) from Thailand. Plants 2023;12:949.
11. Chittrakarn S, Sawangjaroen K, Prasettho S, Janchawee B, Keawpradub N. Inhibitory effects of kratom leaf extract (*Mitragyna speciosa* Korth.) on the rat gastrointestinal tract. J Ethnopharmacol 2008;116:173-8.
12. Chittrakarn S, Radenahmad N, Kaewsara S, Udomuksorn W, Keawpradub N, Phukpattaranont P. Gastroprotective effects of methanolic extract of kratom leaves on gastric ulcer and reflux esophagitis in rats. Songklanakarin J Sci Technol 2018;40:258-63.
13. Haniadka R, Saldanha E, Sunita V, Palatty PL, Fayad R, Baliga

MS. A review of the gastroprotective effects of ginger (Zingiber officinale Roscoe). Food Funct 2013;4:845-55.

14. Gupta RA, Motiwala MN, Dumore NG, Danao KR, Ganjare AB. Effect of piperine on inhibition of FFA induced TLR4 mediated inflammation and amelioration of acetic acid induced ulcerative colitis in mice. J Ethnopharmacol 2015;164:239-46.

16. El-Abhar HS, Hammad LNA, Gawad HAS. Modulating effects of ginger extract on rats with ulcerative colitis. J Ethnopharmacol 2008;118:367-72.

17. Kim CS, Kawada T, Kim BS, Han IS, Choe SY, Kurata T, et al. Capsaicin exhibits anti-inflammatory property by inhibiting IkB-a degradation in LPS-stimulated peritoneal macrophages. Cell Signal 2003;15:299-306.