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Formulation of Capsuled Herbal Mixture Using *Zingiber officinale* and *Labisia pumila* For Postnatal Care

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Abstract: Postnatal period is the most critical phase as the tendency for the mother specifically to get illness during the postpartum period is high. In Malaysia, Ministry of Health (MOH) has established the traditional and complementary medicine (T&CM) to show their support and commitment towards herbal medicine. The aims of this study are to formulate herbal mixture for herbal capsules production based on the traditional knowledge from Temuan community, to analyze phytochemicals of the herbal mixture and to develop herbal medicine in the form of capsules. There were five formulations of *Zingiber officinale* (Ginger) and *Labisia pumila* (Kacip Fatimah) mixtures which had been designed by Design Expert software. Total flavonoid content and antioxidant content of the herbal mixtures were analyzed by using aluminum chloride colorimetric assay and DPPH assay, respectively. The result shows that the mixture with Formulation 3 (ratio 1:3 of *Z. officinale* to *L. pumila*) contained high flavonoid content while, Formulation 1 (ratio 1:1 of *Z. officinale* to *L. pumila*) contained high antioxidant content. Hence, those mixtures were being used to be developed as herbal medicine in the form of capsules using capsule filling machine. The moisture content and disintegration test of the capsules were evaluated for their quality control.

Keywords: postnatal care, herbal capsule, flavonoid, antioxidant, *Zingiber officinale, Labisia pumila*

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1. Introduction

In contemporary times, herbal medicine remains a major component of the primary healthcare in many rural African and Asian communities. Many herbs and herbal recipes have a long traditional history of folk uses and claims of health benefits. Scientific research has shown that herbal medicines contain complex chemical compounds that are responsible for the pharmacological activities such as reducing blood pressure, which corresponds to health benefits and/or toxicity that they elicit [1].

Traditional postnatal practices use herbal medicines as the treatment for any ailment during confinement. Various methods are used including use of spices, medicinal plants and animals; physical treatment such as massage, suction therapy and circumcision; as well as spiritual treatment such as recitation and performing prayers [2]. Herbal processing is needed in making postnatal herbs from plants which make it more difficult and time-consuming. The development of herbal medicine in postnatal tradition and ritual in modern form which is herbal capsule is more practical and easier to be consumed nowadays since the herbal preparation is in ready-made product.

2. Materials and Methods

2.1 Materials

Zingiber officinale (Ginger) rhizomes, *Labisia pumila* (Kacip Fatimah) leaves, distilled water, Whatman filter paper, aqueous methanol, aqueous sodium nitrite solution, aluminum chloride solution, sodium hydroxide solution, UV visible spectrophotometer, DPPH, methanol, ascorbic acid, capsules, MX-50 Moisture Analyzer, disintegration tester by Tianjin Guoming Medicinal Equipment.

2.2 Herbal Formulation

The powder of *Z. officinale* (Ginger) rhizomes and *L. pumila* (Kacip Fatimah) leaves were obtained from the supplier. For the herbal formulations development, the plant sample were mixed in various proportions which had been designated using commercial statistical software package that is Design Expert 6.0.4. (Table 1). Infusion extraction were done where 100 mL of distilled water were heated up to 100°C. Then, 1 g of mixture powder were introduced into the 100 mL of distilled water.. The solution was cooled at room temperature and filtered using Whattman filter paper into a beaker.

2.3 Total Flavonoid Content

Total flavonoid content of herbal mixture were determined using aluminum chloride colorimetric method [3]. The 300 µL of plant extract were mixed with 3.4 mL of aqueous methanol (30%) to obtain a clear solution. Then, 150 µL of aqueous sodium nitrite solution (0.05 M) was added followed by 150 µL aluminum chloride solution (0.3 M). After 5 min, 1 mL sodium hydroxide solution (1 M) was added. The content was mixed well and measure its absorbance at 506 nm on a UV visible spectrophotometer against blank (distilled water). Similarly, a calibration curve of rutin was obtained for concentration ranging from 40 µg/mL to 200 µg/mL. The total flavonoid content of each extract was expressed as µg of rutin equivalent (RE) per mL and calculated using the Equation 1 of rutin equivalent (µg/mL). The content of total flavonoid was calculated using Equation 2.

$$y = 0.0004x + 0.0071 \quad Eq.1$$

$$C = C \times \frac{V}{M} \quad Eq.2$$

2.4 Antioxidant Content

Antioxidant content was determined by DPPH free radical scavenging [3]. The stock solution of the radical, prepared by dissolving 24 mg DPPH in 100 mL methanol and it was kept in a refrigerator until further use. The working solution of the radical was prepared by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 (±0.02) at 517 nm [4]. In a test tube, 3 mL DPPH working solution were mixed with 100 µL plant extract. Then, the solution were incubated for 30 minutes before measuring its absorbance at 517 nm. A standard solution was prepared using the similar method by placing the plant extract with ascorbic acid. The percent antioxidant or radical scavenging activity was calculated using Equation 3.

$$Antioxidant\ activity\ (\%) = [(Ac - As)/Ac] \quad Eq.3$$

2.5 Encapsulation Process

Empty capsule size 0 was purchased online. The powder of *Z. officinale* and *L. pumila* were taken for preparation by filling into the empty capsules. Empty 0 size capsules were placed into the holes of the capsule filling machine by hand with the bodies of the capsule fitting snugly into the plate. The caps of the capsules were removed, then the powder were placed onto the surface of the body plate containing the capsule body and spread with a spreader card so that it flowed into the empty capsule bodies. Alternating gentle shaking of the plate was used to remove any air pockets, and the spreading of more material into the capsule body. Once filled to the brim, the cap of each capsule was repositioned over the material filled body of the capsule and the two capsule parts were rejoined using gentle pressure [5].

2.6 Moisture Content

The moisture could enhance the microbial contamination of the herbal capsule. The shell of the capsules was removed and the moisture level of the contents of the capsules were performed using the MX-50 Moisture Analyzer instrument by A&D. The moisture content of five capsules were evaluated for each formulations (1:3 and 1:1) and the average value was taken.

2.7 Disintegration test

The disintegration testing as a performance test is critical in the pharmaceutical development [6]. The capsules were undertaken using disintegration tester by Tianjin Guoming Medicinal Equipment at 37 ± 2°C with distilled water as disintegrating medium. The time taken for all six capsules to disintegrate leaving only remnants of gelatin shell on the mesh was recorded. The procedure was repeated thrice and the mean disintegration time was calculated.

3. Results and Discussion

3.1 Formulation of herbal mixture

Table 1: Design layout and herbal proportions

Formulations	Ratio	Factor 1 (%) *Zingiber officinale*	Factor 2 (%) *Labisia pumila*
1	1:1	50	50
2	1:0	100	0
3	1:3	25	75
4	0:1	0	100
5	3:1	75	25

There were five formulations of herbal mixture that have been designed by the software (Table 1). The ratios represent the percentage of the *Z. officinale* and *L. pumila* powder to form a total mixture of 1g of powder sample. There was ratio that contain only single plant which was *Z. officinale* (1:0) or *L. pumila* (0:1) only. 1 to 1 (1:1) ratio is the combination of both plants with equal quantity while, the ratio of 1 to 3 (1:3) or 3 to 1 (3:1) is the mixture of both plant with unequal portion.

3.2 Total Flavonoid Content (TFC)

Table 2: TFC value of different formulation

Ratio	Formulation		Total Flavonoid Content (TFC) (µg RE/mL)
Zingiber officinale: Labisia pumila	*Zingiber officinale (%)*	*Labisia pumila (%)*	
1:1	50	50	11.89 ± 0.38
1:3	25	75	13.73 ± 0.75
3:1	75	25	8.98 ± 0.433
0:1	0	100	15.89 ± 0.52
1:0	100	0	10.98 ± 0.87

Formulation with ratio 0 to 1 (0 *Z. officinal*e to 1 *L. pumila*) have the highest TFC which is 15.892 µg RE/mL while formulation with ratio 3 to 1 (3 *Z. officinal*e to 1 *L. pumila*) have the least TFC value which is 8.975 µg RE/mL. However, among the formulations with mixture of both herbal plant species the highest TFC is the formulation with ratio 1 *Z. officinal*e to 3 *L. pumila* (13.725 µg RE/mL). Hence, it can be conclude that *L. pumila* influence the TFC value of the herbal formulations. These can be proven that the value of TFC become higher when the amount of *L. pumila* in the formulation is higher.

Moreover, a few studies have also showed that variety of flavonoids could produce significant anti-inflammatory activities for instance rutin, hesperidin and biflavonoids [7,8]. Therefore, it can be also be suggested that the mixture of the extract possesses high concentration of flavonoids and thus gave anti-inflammatory effect.

3.3 Antioxidant content

From the analysis of Figure 1, the scavenging effect of the samples were promising especially in the case of the formulation with ratio 1:0 (1 *Z. officinal*e to 0 *Labisia pumila*) extract. This is because, the value of antioxidant activity is close to the value of antioxidant activity of the standard and the highest compared to the other formulations. While, the lowest value of the antioxidant activity among them is the ratio 3:1 formulations (3 *Z. officinal*e to 1 *L. pumila*) extract.

Nevertheless, the formulations with the ratio 1:1 (1 *Z. officinal*e to 1 *L. pumila*) extract has the highest scavenging effect compared to the other formulations which contain both mixture of plant samples.

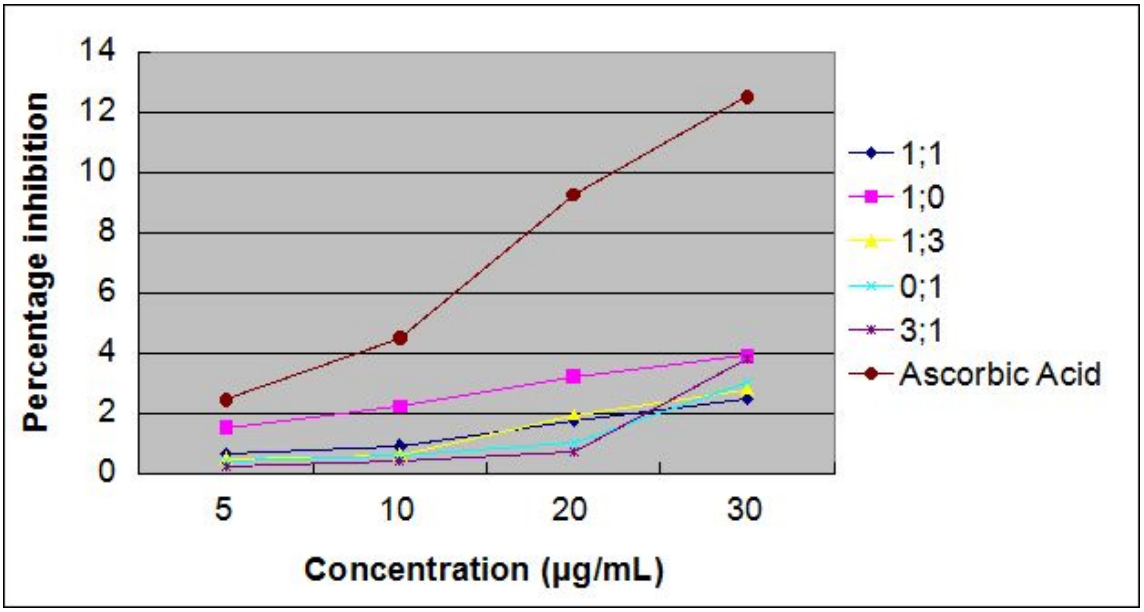

Figure 1: Percentage inhibition graph of DPPH free radical scavenging of plant extract and standard

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule [9]. The main characteristic of an antioxidant is its capability to trap free radicals. Antioxidant compounds scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases [10] such as asthma, inflammatory, arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis [11].

3.4 Physical Properties Test

The physical properties tests conducted for the herbal mixture capsules were moisture content and disintegration test. The average moisture content of herbal mixture capsules with the ratio 1 *Z. officinal*e to 3 *L. pumila* (1:3) was 0.54% while, the herbal mixture capsules with ratio 1 *Z. officinal*e to 1 *L. pumila* (1:1) was 0.56%. These value indicates that there is a less chance of microbial growth. The lower the tendency for the capsules to be contaminated by the microbe, the higher the life span of the capsules.

The disintegration test of the capsules was performed by using disintegration tester to indicate the length of time taken by the herbal mixture capsules to disintegrate or dissolve in the gastrointestinal tract of the consumers. The average time taken of the capsules to disintegrate for both herbal mixture with ratio 1 *Z. officinal*e to 3 *L. pumila* (1:3) and 1 *Z. officinal*e to 1 *L. pumila* (1:1) were 15 minutes

for all six capsules. The disintegration process involves two processes in which the intact herbal mixture capsules are disintegrated into granules form and later on are disaggregrated for faster dissolution rate. Once these processes are completed, the herbal drugs dissolved in the solution and are absorbed into the human body [11].

4. Conclusion

The herbal plants contain various chemical constituents such as flavonoids and antioxidants which could help to medicate many diseases including the ailments or complications during childbirth period. The herbal mixture with the ratio 1 *Z. officinal*e to 3 *L. pumila* extract have the highest flavonoid content while, the highest antioxidant content is the herbal mixture with the ratio 1 *Z. officinal*e to 1 *L. pumila* extract. Thus, it shows that to obtain both high in flavonoids content and antioxidants content in one formulation is challenging but possible as the mixture of herbs may effect the initial value of phytochemicals of the individual plant. High in flavonoid content in the herbal plant aid the inflammations due to the childbirth process. Whereas, high in antioxidant help to inhibit the oxidative damage during their postnatal period. Physical properties of the capsules such as moisture content and disintegration test were evaluated to ensure their efficacy of the drug for the consumer. This research help to protect and conserve diversity of plants from being threaten as it contains therapeutic properties which is very helpful for human being.

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