

Review

Phytoestrogenic property of *Labisia pumila* for use as an estrogen replacement therapy agent

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Labisia pumila (LP), also known as Kacip Fatimah has been used by Malay women for generations for conditions related to menopausal symptoms. Though, there has been no scientific-based evidence for its efficacy as an estrogen replacement therapy (ERT), LP's use continues to be on the rise. This could be seen with increase in commercially available supplements which claims that taking the pills can help eliminate menopausal symptoms. To date, most researches only show us the difference in LP's effect on certain metabolic activities with that of non-treated or estrogen-treated. However, the mechanism which brought about such changes is still unknown. This is because the pathways which are affected by LP or the cross-talk with other estrogenic pathways are still unknown. Perhaps in future, these are areas in which research on LP could be focused on.

Key words: *Labisia pumila*, Kacip Fatimah, traditional medicine, estrogen replacement therapy.

INTRODUCTION

Labisia pumila (LP), from the family of Myrsinaceae, is a sub-herbaceous shrub that is found to be growing wildly on the forest floor of peninsular Malaysia. In Malaysia, it is known locally as "Kacip Fatimah" and has been used by Malay women for generations. Traditionally, the plant will be boiled, either alone or in decoction with other herbs before being consumed (Burkill, 1935). According to Stone (1998), there are three varieties of Kacip Fatimah that exist in Malaysia: LP var. *Alata* (LPva), LP var. *Lanceolata* (LPvl) and LP var. *Pumila* (LPvp) (Figure 1). They are distinguished from each other by their petiole and leaf characteristic as well as their usage (Karimi et al., 2011). LPva, LPvl and LPvp have a winged red vein petiole, long and non-winged petiole and marginate petiole with ovate leaf blade shape, respectively (Stone, 1988). LP products are available commercially as health supplements to alleviate menopausal symptoms, such as

hot flushes and night sweats. These menopausal symptoms occur due to lack of estrogen (Liu et al., 2001) in the body and explain why women undergo estrogen replacement therapy (ERT) (Norhaiza et al., 2009).

PHYTOESTROGENICITY OF LP

Due to LP's exclusive use in women for applications related to ERT, it has long been speculated that they exhibit phytoestrogenic activity. Phytoestrogens were postulated to have a pair of hydroxyl group and a phenolic ring which is required for binding to the estrogen receptors (ER) (Mense et al., 2008). As reported by Husniza, (2002), water extract of LP could inhibit estradiol (E₂) binding to antibodies raised against E₂, showing similar effect as estrone and estriol. On the other hand, Jamal et al. (2003) found out that ethanol extract of LPva root exhibits a weak estrogenic activity at 10 to 50 mg/ml in an *in vitro* assay in Ishikawa cells, but none was observed from the leaf extract. Interestingly, no activity was seen in the aqueous extract from the same roots (Jamal et al., 2003). To date, no one had attempted to study the bioactive components of LP.

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Abbreviations: LP, *Labisia pumila*; ERT, estrogen replacement therapy.



Figure 1. *LPvp* (Herbarium Laboratory, Biological Schools, Universiti Sains Malaysia).

This warrants a more in-depth analysis, particularly, using biochemical assays in the hope to identify novel bioactive compounds. The wide spectrum of activities exhibited by *LPva* was believed to be due to the flavonoids and phenolic contents extracted in water and organic solvents (Norhaiza et al., 2009; Chua et al., 2011). Both flavonoids and phenolics have remarkable pharmacological activity example anti-inflammatory, anti-oxidant and anti-cancer activity (Norhaiza et al., 2009; Chua et al., 2011; Karimi et al., 2011; Fazliana et al., 2011). *LPva* extract also induces apoptosis and reduces bacterial load of *Escherichia coli* in urinary tract infections of postmenopausal women and thus has apoptosis-inducing activity (Fazliana et al., 2011). Besides that water extract of *LP* has also been reported to have anti-photoaging activity on human dermal fibroblasts by protecting them from cell damage caused by ultraviolet (UV) irradiation (Choi et al., 2010). The estrogenic activity may differ based on the different classes of flavonoids, phenolic content, the variety and anatomy of *LP* plant. Nevertheless, total phenolics and flavonoids were found

to be the highest in the leaf extract and the lowest in the stem. Karimi et al. (2011) reported that the leaf extract of *LPva* has the highest amount of phenolics, while leaf extract of *LPvp* has the highest levels of flavonoids.

Till present, there are limited journals published in the area of evaluating *LP*'s estrogenicity, mode of action, efficacy and toxicity levels, as well as its possible interactions with other compounds. This is because the mechanism pathway which results in the physiological or metabolic change is still unknown. In spite of the lack of pharmacological data, there is an increase in commercially available *LP* supplements (Ezumi et al., 2006). It is of utmost importance for the pharmacokinetics of these supplements to be thoroughly studied with details on the half maximal efficacy concentration (EC_{50}), half maximal inhibitory concentration (IC_{50}) or median lethal dose (LD_{50}) and its possible side effects. Thus, it is important to first evaluate the bioactive compound for toxicology studies on a molecular level. At present, our main focus is to elucidate the bioactive compound through the binding assays and detection of signaling molecules.

PHYTOESTROGENS AS ESTROGEN REPLACEMENT THERAPY

Menopause has been associated to menopausal symptoms, decreased uterine weight, increased cardiovascular risk and osteoporosis (Umland et al., 2000) and thus, ERT aims to overcome these symptoms. There are various types of ERT available commercially which range from pills, cream to gel.

All these contain estrogen in the form of micronized E_2 , conjugated equine estrogen (CEE) and esterified estrogen (NIH, 2005). CEE, which is a complex natural urinary extract of pregnant mare's urine, is the most commonly used form of estrogen (Bhavnani, 2003). Bioassays and ER binding studies indicate that all estrogens in CEE are biologically active and can interact with recombinant human ER- α and ER- β (Bhavnani, 2003).

In 2002, Women's Health Initiative Investigators reported that prempo (a synthetic drug), contains CEE and had resulted in an increased risk of breast cancer, coronary heart disease and stroke, which lead to the termination of a long term clinical trial. These side effects are one of the reasons why dietary phytoestrogens are being promoted as an alternative to synthetic estrogens (Liu et al., 2001; Chlebowski et al., 2003; Overk et al., 2005).

There are also more women who prefer natural approaches to overcome menopausal symptoms (Glazier and Bowman, 2011). Phytoestrogens are divided into various classes that include isoflavones, lignans, coumestans, and stilbenes (Rice and Whitehead, 2006). Epidemiologic data showed strong associations between diets high in foods containing phytoestrogens and a reduction in cardio-vascular disease and

menopausal symptoms (Umland et al., 2000), although, the beneficial effects observed are unique to the Asian population (Glazier and Bowman, 2011). For example, red clover isoflavones (40 mg/day) increases arterial elasticity and improves cardiovascular health (Xu et al., 1998). Meanwhile, genistein and daidzein (soy phytoestrogens) were shown to reverse bone loss in ovariectomized (OVX) rats (Arjmandi et al., 1998). At present, there is little research in humans and the most positive evidence of phytoestrogen came from *in vitro* or animal studies (Glazier and Bowman, 2011).

LP AS A POTENTIAL ESTROGEN REPLACEMENT THERAPY

Research using animal models indicated that *LP* at a dose of 17.5 mg/kg/day could maintain elastic lamellae architecture of OVX rat aorta in a manner similar to ERT and thus, showed a possible role in modulating postmenopausal cardiovascular risks (Al-Wahaibi et al., 2008). In a separate study, both ERT and *LP* (50 mg/kg/day) were shown to suppress weight gain in OVX rats by increasing plasma leptin and decreasing resistin levels in OVX rats (Fazliana et al., 2009), but was not observed in polycystic ovary syndrome (PCOS) rats. In a later study, 17.5 mg/kg *LP* and 64.5 µg/kg E₂ failed to prevent weight gained induced by OVX (Shuid et al., 2011), which was also opposite to the findings of Fazliana et al. (2009). Mannerås et al. (2010) showed that *LP* at 50 mg/kg/day in dehydrotestosterone (DHT)-induced PCOS rats resulted in the opposite effect of increased resistin and decreased mRNA leptin levels, resulting in increased uterine weight. It has been suggested that the different results observed could be due to the different rat models used as *LP* could have acted through different estrogenic mechanisms in both models (Mannerås et al., 2010). However, to date, no study has been done to access the different estrogenic or non-estrogenic mechanisms of *LP* between the different rat models. If *LP* works through estrogenic pathways, increase in resistin level is consistent with *in vitro* studies of adipocytes showing that E₂ stimulates resistin gene expression (Chen et al., 2006). Besides increase in resistin, another similarity between *LP* and E₂ is the decrease in insulin sensitivity in the OVX and PCOS rats (Gonzales et al., 2002; Manneras et al., 2010). On the other hand, a down-regulation of 11 beta-hydroxysteroid dehydrogenase 1 and corticosterone protein levels in both the liver and adipose tissue (Fazliana et al., 2012) were seen.

In the aforementioned studies, *LP* displayed biological effects similar to that of ERT (Ma et al., 2010) and thus demonstrates the potential of *LP* as a natural ERT. Though, *LP* had been claimed to treat menstrual irregularity and function as a post-partum medication (Fazliana et al., 2011), and there is an increasing

evidence for the beneficial effects of phytoestrogens, but it is still too early to recommend the use of *LP* over conventional ERT as the benefits of taking traditional ERT far outweighs that of phytoestrogen consumption (Umland et al., 2000). Also, there is no evidence to conclude that *LP* could reduce menopausal symptoms, cardiovascular or osteoporosis risk. Findings at present do not show the mechanism in which *LP* can act similar to an ERT agent example same signaling pathways or downstream molecular targets. Nonetheless, all these studies have contributed to the limited knowledge on *LP* activity. In order to better understand the result seen in these studies, the mechanism of action for *LP* and the pathways in which it affects have to be identified and compared to that of ERT. *LP* may have a complex mechanism of action, working through hormone-dependent or hormone-independent pathways (Setchell, 1998). In cases where *LP* did not react similarly to estrogen, it could be due to the collection of compounds present in the crude extract. In order to evaluate the possible relation between these compounds, intensive study on the phytochemistry of *LP* and its active compound is required. Overall, data on the estrogenic actions of phytoestrogens are perplexed by many factors, such as the dosage, presence of endogenous estrogens, ages of the subjects and treatment regimes.

CONCLUSION

Although, we acknowledge that *LP* is a popular herb among the Malays in Malaysia, continuous increase in commercial *LP* supplements enables it to open its market to non-Malays in the country. However, despite their widespread use and increasing acceptance, there are limited data on its efficacy as an ERT. Without knowing the components of *LP* and the active component with or without E₂ activity, it would be difficult to perform a comprehensive study on *LP*.

We believe that *LP* should to be subjected to a detailed pharmacological investigation for it to gain widespread acceptance as a natural ERT. Nevertheless, we hope to identify the pure active compound from *LP* that could potentially be used as a natural ERT and to facilitate the identification of pharmacological activities, example in identifying the pathways leading to the metabolic activities observed and possibility of cross-talk between *LP* and E₂ pathways. Overall, we would highlight that this notion is the best way in identification of novel bioactive compounds in *LP* in the effort to create a natural ERT to decrease the side effects from synthetic ERT drugs.

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