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Review article

Therapeutic potentials of *Marantodes pumilum* (Blume) Kuntze var. *alata* as antiresorptive agent in management of postmenopausal osteoporosis

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Abstract

Due to increasing population of the elderly owing to higher life expectancy, osteoporosis in postmenopausal women has become a primary public health problem, posing huge economic and social burden today. Globally, approximately 200 million women are affected by this condition worldwide. One of every 3 women above 50 years was reported to experience fractures secondary to osteoporosis. Out of 8.9 million fractures (hip: 1.6 million, forearm: 1.7 million, clinical vertebral: 1.4 million) recorded annually, 61% occurred in women. Hormone replacement therapy (HRT) using estrogen has been a valuable treatment option in the management of postmenopausal osteoporosis. Due to adverse side effects such as breast, colorectal and endometrial cancer, pulmonary embolism and stroke associated with long term use of estrogen, HRT is now reserved for treatment of pressing postmenopausal symptoms such as hot flashes and vaginal dryness. Currently, a wide range of other agents such alendronate, raloxifene, calcitonin, vitamin D and calcium are recommended and used as alternatives. But, like HRT, these agents are also known to be associated with debilitating side effects that often hamper compliance to drug regimen. Thus, alternative and complimentary therapies for treating postmenopausal osteoporosis are being sorted for. *Marantodes pumilum* (Blume) Kuntze var alata, a phytoestrogen-rich herb, is one of the numerous natural agents that have been reported to be useful in protecting the bone against osteoporosis and is currently recommended for management of postmenopausal symptoms. Researchers have reported protection of estrogen deficient animals against osteoporosis. In this article, the potentials of *M. pumilum* to be used as a safe alternative to estrogen in managing postmenopausal osteoporosis was explored by reviewing relevant published data on its osteoprotective and related pharmacological activities from reputable and reliable databases.

Key words: *Marantodes pumilum* (Blume) Kuntze, kacip fatimah, osteoporosis, osteoprotection, phytoestrogens, *Labisia pumila*.

1. Introduction

Osteoporosis is a degenerative disease of the bone that is said to occur when standard reference bone mineral density (maximum bone mineral density of young adult female) falls by more than 2.5 standard deviation (SD). It causes loss of bone mass and degeneration in micro-architecture of bone tissue leading to increased bone fragility and fracture susceptibility (World Health Organization, 2004; Consensus Development Conference, 1993). It is caused by an imbalance in bone remodeling process where the rate of bone resorption by osteoclast cells is higher than the rate of bone formation by osteoblast cells (Hui, 1998). As the ageing population of men and women increases, osteoporosis has become an important public health problem, causing huge economic and social burden (Nancy, 2006). Risk factors associated with osteoporosis are medical, behavioral, nutritional and genetic factors such as race, sex, age, body size, previous fractures, chronic inactivity and low body weight (Rao and Rao, 2013).

Statistical records have revealed higher incidence of postmenopausal osteoporosis in women than in men of the same age group. About 30% of women and 12% of men, at a period of their lifetime, will experience osteoporosis (Laura and Robert, 2012). It was reported that 1 out of every 3 and 8 women and men, respectively, above 50 years would experience osteoporosis-related fractures (International Osteoporosis Foundation, 2009; Cooper and Melton, 1992). Bone mass in women is lower than that of a male counterpart at 50 years by one-third (Thomsen, 1986); this may be due to a combination of lower initial adult bone mass and a more rapid rate of bone loss in elderly women than in men (Ismail, 1997). It has been shown that estrogen deficiency is responsible for decrease osteoblast activity and a subsequent decrease in bone mass (Garnero *et al*., 1996). Thus, the higher incidence of osteoporosis in women can be logically explained by the link between estrogen deficiency and bone loss in women after menopause.

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Vertebral fractures are the most common complication of osteoporosis accounting for about 47% of total fractures due to osteoporosis. They are usually painless and accompanied with height loss, low quality of life, and respiratory problems as well as diminished survival rate (Scane *et al*., 1999; Keating *et al*., 2000; Lau *et al*., 2008). Hip fractures, which account for 17% of fractures due to osteoporosis, are the most fatal complication of osteoporosis with a mortality rate of 37.5% (Chang *et al*., 2004; Jiang *et al*., 2005).

Treatment of osteoporosis in postmenopausal women previously relied on long-term use of estrogen (Al-Azzawi, 2008). Studies have shown that estrogen, as a replacement therapy (ERT), significantly reduced and increased loss of bone and bone density, respectively, resulting in reduction in risk of osteoporotic fracture (Keating *et al*., 2000). Due to reports of adverse side effects such as cancer (breast, endometrial and colorectal), pulmonary edema, stroke, hip fracture and death, this treatment approach has been reserved (Cynthia *et al*., 2012; Rossouw *et al*., 2002). It is now recommended for use in the treatment of menopausal symptoms such as hot flashes and in management of postmenopausal osteoporosis only when strict conditions of safety are satisfied (National Osteoporosis Society Position Statement, 2010; Furness *et al*., 2012). A number of other drugs such as alendronate (bisphosphonates), raloxifene (selective estrogen receptor modulators, SERM), PTH-34 (anabolic therapies), calcium and vitamin D, calcitonin, denosumab (human monoclonal antibodies) are now recommended for treating postmenopausal osteoporosis. However, none of these alternatives have shown to match the clinical outcome of ERT and is without disturbing side effects (Rao and Rao, 2013). Thus, there is an increasing demand for a safer alternative to ERT for treatment and possible prevention of osteoporosis in postmenopausal women (Rees, 2011). In this light, investigations have been carried out on a number of natural agents such as soy and blueberry (Devareddy *et al*., 2006; Devareddy *et al*., 2008), *Achyranthes bidentata* (He *et al*., 2010), *M. pumilum* (Shuid *et al*., 2011) and virgin coconut oil (Soelaiman *et al*., 2013).

This article is aimed at reviewing relevant research data on the osteoprotective and related pharmacological attributes of *M. pumilum* (MPva). It explores the chances of MPva to be used as alternative or complementary medicine to estrogen in management of postmenopausal osteoporosis, and also provides platform for further researches. Published literatures on the uses, efficacy, safety and possible mechanism of action of *M. pumilum* in postmenopausal osteoporosis and related conditions from reliable databases such as PubMed, Cochrane library, Scopus, Web of Science and Natural Medicines, ResearchGate, academia edu. and many more were reviewed. Search phrases such as 'uses of *M. pumilum*', 'osteoprotection', 'bone biomarkers', 'bone remodeling', 'anti-inflammatory effects', 'antioxidant effects' were used .

2. *Marantodes pumilum* (Blume) Kuntze

M. pumilum (Blume) Kuntze (synonyms: *Labisia pumila* (Blume) Fern.-Vill; *Labisia pumila* (Blume) Mez; *Ardisi apumilum* Blume) is a herb plant belonging to family Myrsinaceae (The Plant List, 2010). It is known as the queen of herbs in Malaysia where it is popularly called Kacip Fatimah. It is also traditionally known as: *seluso Fatimah, Kacit Fatimah, Tadah Malahari, Mata pelanduk, Rimba and Sang Koh*. It grows widely at 80-100 meters above sea level in the rain forest of Malaysia, Indonesia, Thailand, Indochina, the Philippines and New Guinea. Its leaves have a characteristic winged petiole with red veins (Burkill, 1996). Of the eight described varieties of *M. pumilum*, 'var alata' variety is the most widely studied (Stone, 1998). Traditionally, hot water decoction of the plant parts (roots and leaves) or even the whole plant is taken orally to treat many ailments relating to women health such as menstrual irregularities, female reproductive health problems and as libido enhancement (Runi, 2000). It is given to pregnant women, 1 to 2 months before birth, to induce and expedite labour at term (Sunarno, 2005). Post-partum, it is used to shrink the uterus, abdominal muscles and regain strength. Nowadays, it is used as tonic to treat pre and postmenopausal symptoms. It is also used for treating rheumatism, gonorrhea and excessive gas from the body.

Phytochemical studies have revealed the presence of phytoconstituents that are mainly phenolic in nature (flavonoids and phenolic acids). The nature and composition of these phytoconstituents have been shown to vary according to plant parts (Table 1). Flavonoids such as quercetin, myricetin and kaemferol have found usefulness as anti-inflammatory, antioxidants, anticancer, antimicrobial as well as antiresorptive agents in modern medicine while phenolic acids such as gallic acid, vanillic acid and syringic acid have received attention due to their ability to protect against cancer and heart disease (Yamamoto and Gaynor, 2001; Tsao and Deng, 2004). Due to the ability of these phytochemicals to elicit estrogen-like biological activities (Jamia *et al*., 2003), they have been widely referred to as phytoestrogens.

Table 1: Phytochemicals of *Marantodes pumilum*

Plant Part	Phytochemical(s) identified	Reference
Leaves and roots	13,28-epoxy-oleanane glycoside (Z)-5-(pentadec-42 enyl)-resorcinol, (Z)-5-(pentadec-82 -enyl)-resorcinol and (Z)-5-(pentadec-102 -enyl)-resorcinol	Jamia and Houghton (1999)
Leaves	Anthocyanins, ascorbic acid and β-carotene	Norhaiza *et al.* (2009)
Leaves, Stem and roots	Gallic acid, caffeic acid, pyrogallol Kaempferol, myricetin, naringin quercetin, rutin, Daidzein and genistein	Karimi *et al.* (2011)
Leaves	Quercetin, myricetin, kaempferol Catechin, epigallocatechin, Salicylic acid, syringic acid and vanillic acid	Chua *et al.* (2011)
Leaves, stem and roots	Ardisiacrispin A, ardisicrenoside B, ardisimamilloside H, arabinopyna-nosyl cyclamiretin A Irisresorcinol, belamcandol B and demethylbelamcan daquinone B	Avula *et al.* (2011)
Roots	Demethylbelamcandaquinone B and fatimahol	Ali and Khan (2011)

Pharmacological studies carried out on *M. pumilum* have reported a wide range of biologic activities in laboratory animals. These include: estrogenic (Husniza, 2002; Fazliana *et al*., 2009; Ayida *et al*., 2007), antioxidant (Norhaiza *et al*., 2009), antiasthmatic (Ekeuku and Okechukwu, 2012), anti-inflammatory (Choi *et al*., 2010), antihistaminic, antinociceptive and antiulcer (Okechukwu and Marunga, 2011, Okechukwu and Ekeuku, 2012), cytotoxic (Al-Mekhalafi *et al*., 2012), antihyperlipidemic (George *et al*., 2014) skin protective (Chua *et al*., 2011), weight reduction (Pandey *et al*., 2104) and osteoprotective (Shuid *et al*., 2011) properties.

3. Osteoprotective properties of MPva

3.1 Effects on bone microarchitectural morphometry

Quantitative morphometry of bone architectural properties gives an insight into the amount, size, shape and connectivity of trabecular and cortical bone tissues, and have been used to predict bone strength (Bouxsein *et al*., 2010). A study was conducted to investigate the effects of aqueous crude extract of MPva root on bone histomorphometric parameters, *i.e*. static, dynamic and structural bone parameters of the femur of ovariectomized rat after 2 months treatment period (Fathilah *et al*., 2012a). It was revealed that *M. pumilum* group recorded significantly higher levels of trabecular thickness (Tb.Th), trabecular number (Tb.N) and lower trabecular separation (Tb.Sp) when compared with healthy (sham-operated) and ovariectomized control groups. Similarly, on the dynamic parameters, the group that received *M. pumilum* recorded higher double-labeled surface (dls/BS), mineral apposition surface (MAR), bone formation rate (BFR/BS) and mineralizing surface (MS/BS) parameters than the sham-operated and ovariectomized control groups. On the static parameters, *M. pumilum* group showed significantly higher levels of osteoblast surface (ObS/BS), osteoid surface (OS/BS) and osteoid volume (OV/BV) parameters when compared with ovariectomized control group. When *M. pumilum* group was compared with the group that received 64.5 µg/kg/day of estrogen, no significant difference in the structural, static and dynamic parameters was seen.

3.2 Effects on bone biomechanical strength properties

Overall strength of the bone depends not only on bone mineral densities, but also on quantitative morphometry of its micro-architectural structures of both trabecular and cortical bone. The predictive value of mineral density results when complemented with that of structural morphometric parameters are more reliable in prediction of fracture and diagnosis of other bone conditions (Ulrich, 1999). In a mechanical strength study, biomechanical strength parameters (stress, strain and young's modulus) of femur bone of ovariectomized rats were investigated after 8 weeks supplementation with aqueous root extract of *M. pumilum* (Fathilah *et al*., 2012b). The study revealed that the biomechanical strength parameters of the femur bones were significantly higher in the group that received 17.5 mg/kg/day *M. pumilum* when compared with ovariectomized control group. However, when compared to the group that received 64.5 µg/kg/day estrogen, *M. pumilum* group showed no significant difference in bone strength parameters. In another study, significant improvement in bone mechanical strength parameters, maximum load, displacement, stress and young's modulus, were seen in rats supplemented with *M. pumilum* for nine weeks (Nadia *et al*., 2015).

3.3 Effects on bone turnover markers

In bone remodeling process, biochemical markers of bone resorptive and formative processes are often released into blood stream. Measuring these markers can also be used to provide useful clinical information on bone turnover rate, bone health and as a complimentary diagnostic tool to BMD (Johnell *et al*., 2002). In a study published in 2011 (Shuid *et al*., 2011), bone biomarkers and total calcium contents were investigated in eight (8) weeks *M. pumilum* root extract supplemented ovariectomized rats. The study revealed significantly higher levels of osteocalcin (a bone formation marker) and lower C-terminal telopeptide of type 1 collagen, CTX-1 (a bone resorption marker) in the group that received 17.5 mg/kg/day *M. pumilum* than in ovariectomized control group. When *M. pumilum* group was compared with estrogen treated control group, no significant difference in resultant effects on bone biomarkers was observed. However, results from the assay of bone calcium content revealed the failure of *M. pumilum* to preserve bone calcium content as much as estrogen treatment

3.4 Effects on oxidative status markers of bone

Epidemiological evidences from human and animal studies have reported that ageing and increased reactive oxygen species (RO) from oxidative stress were responsible for bone loss (Manolagas and Parfitt, 2010). Oxidative stress due to estrogen deficiency may increase bone resorption *via* the activation of nuclear factor kappa B(NF-kB), a key player in osteoclastogenesis (Iotsova *et al*., 1997). Antioxidants such as tocopherol have been reported to prevent bone loss in oxidative stress condition (Ima Nirwana *et al*., 2012; Norazlina *et al*., 2007). An *in vitro* study by Mohamad *et al*. (2009) showed that *M. pumilum* leaf extract possessed good antioxidant property comparable to vitamin C. This antioxidant activity has been attributed to its phenolic and flavonoids content (Huang *et al*., 2000). In another study (*in vivo*) carried out by Nadia and Nazrun (2014), effects of *M. pumilum* on oxidative status of estrogen-deficient rats were determined by measuring bone oxidative markers (superoxide dismutase, SOD, glutathione peroxidase, GPx and malondialdehyde, MDA). In this study, *M. pumilum* group showed significantly higher level of antioxidant enzyme, SOD, when compared with ovariectomized control and estrogen treatment groups after 6 weeks treatment period. However, after 9 weeks treatment period, the *M. pumilum* group showed significantly higher level of SOD when compared to ovariectomized control group only. GPx level was also found to be significantly higher in *M. pumilum* group than in ovariectomized control group after 9 weeks of treatment. The *M. pumilum* group, like estrogen treatment group, was found to have significantly lower MDA level than the ovariectomized control group after 6 and 9 weeks treatment period.

3.5 Anti-inflammatory and immunomodulatory properties

Inflammatory processes have been shown to cause osteoporosis by influencing bone turnover rate. Certain pro-inflammatory cytokines, *e.g.*, TNF-alpha and interleukins (IL-1,6,11,15,17) that play critical roles in normal bone remodeling and pathogenesis of osteoporosis are released during the course of inflammation (Manolagas and Parfitt, 2010; Jilka, 1998; Lorenzo, 2000). Such mediators have been shown to promote bone resorption by influencing osteoclastogenesis through self-renewal and inhibiting programmed death of osteoclasts progenitors (Lerner and Ohlin, 1993). In addition, increased level of COX-II, which was proven to

stimulate production of prostaglandin (PGE2) leading to an increase in bone resorption (Bakker *et al*., 2003) was found to be expressed after stimulation with pro-inflammatory cytokines such as IL-1 and TNF-α (Crofford, 1997). Several bone resorption studies have shown that a fall in estrogen level in postmenopausal women causes significant production of pro-inflammatory mediators that stimulates local inflammation in the bone (Jilka, 1998). Suppression of these mediators could prevent bone loss commonly seen in menopausal women (Richards *et al*., 2006; Kimble *et al*., 1995). Aqueous crude extract of *M. pumilum* have been reported to possess anti-inflammatory effect (Sanusi *et al*., 2013) and markedly inhibited tumor necrosis factor alpha (TNF-α) and cyclooxygenase II (COX-II) production and expression, respectively (Arron and Choi, 2000). These properties of *M. pumilum* were also thought to contribute to its osteoprotective effect and could possibly explain its mechanism of action.

3.6 Effects on bone remodeling genes

Resorption and formation activities in bone remodeling process have been shown to involve several genetic factors. Administration of serum receptor activator of nuclear factor kappa-B ligand (RANKL) to mice have been shown to promote activation and growth of osteoclast cells leading to increased resorption and development of osteoporosis by binding to RANK receptors located on osteoclast precursors and promoting its differentiation and activation into mature osteoclast cells that control bone resorption (Lacey *et al*., 1998; Teiteilbaum, 2000). Another factor, Osteoprotegerin (OPG) produced by osteoblast cells along with RANKL was also reported to regulate bone resorption. OPG acts as antiresorptive decoy receptor that binds to RANKL thereby preventing it from binding to main RANK receptors. As a result, it inhibits osteoclast differentiation and its resorptive activities. The process of osteoclastogenesis also requires another factor expressed by osteoblasts called macrophage-colony stimulating factor (MCSF). This factor binds to the MCSF receptors in the osteoclast cells and stimulates osteoclastogenesis through an unclear mechanism. Numerous factors such as bone morphogenetic protein-2 (BMP-2) are known to affect osteoblast differentiation and activity (Riley *et al*., 1996). Estrogen used for protection of bone density have exhibited regulation of expressions of genes of these factors (Bord *et al*., 2003).

Aqueous crude extract of *M. pumilum* root was reported to protect bone of animals by reversing the ovariectomy-induced changes in the RANKL, OPG and BMP-2 gene expressions (Fathilah *et al*., 2014). The study revealed that the *M. pumilum* treatment group recorded significant increase in expression of OPG and BMP-2 genes and decrease in expression of RANKL gene when compared with ovariectomized group. However, when compared to sham-operated and estrogen-treatment control groups, no significant difference was seen in expressions of these genes in *M. pumilum* treatment group. There was also no significant change in MCSF gene expressions between all treatment groups.

3.7 Role as phytoestrogens

Phytoestrogens are naturally occurring phytochemical substances with estrogenic nature. Like estrogens, they are known to act as female sexual hormone by displacing estradiol binding to antibodies raised against estradiol and are thus called estrogen receptor modulators, ERM (Husniza, 2002). In an *in vitro* study using human endometrial adenocarcinoma cell line, phytochemicals of *M. pumilum* elicited estrogen-like responses by competing with radiolabelled estradiol for estrogen receptor (Jamia *et al*., 2003). In another study, similar to estrogen (Premarin®), 17.5 mg/kg dose of aqueous extract of *M. pumilum* caused elevation of blood levels of estrogen and testosterone, and suppression of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in rats when compared with untreated ovariectomized control group (Wahab *et al*., 2010). Thus, *M. pumilum* is believed to contain phytoestrogens.

In a number of studies, phytoestrogens have been reported to exert osteoprotective effects in human models. In a randomized clinical trial using synthetic phytoestrogens, ipriflavone, bone effects of phytoestrogens were evaluated in postmenopausal women. After 12 and 24 months treatment period, 1.1 % and 1.2 % improvement in bone density, respectively, were seen in the lumbar spine when compared to placebo group that showed 1.8 % and 3.7 % bone loss after 12 and 24 months treatment, respectively (Gambacciani *et al*., 1997). Therefore, estrogenic properties of *M. pumilum* elicited by its phytoestrogenic content could be responsible for its osteoprotective action.

4. Safety profile

4.1 Animal studies

Frequencies measured from micronucleus of cells can be used as index for detecting chromosome breakages and loss *in vivo* (Shahrim *et al*., 2006). In a cytogenetic toxicological study using bone marrow cells from rat and measuring frequencies from their micronucleus (Shahrim *et al*., 2006), *M. pumilum* extract showed no significant genotoxicity at various doses used. In this study, no significant increase in micronucleated polychromatic erythrocytes at dose levels of 100, 700 and 2000 mg/kg was observed after 48 hours treatment period and 24, 48 and 72 hours harvest time point when compared to mitomycin C (0.75 mg/kg) and placebo.

In a sub-acute toxicity study carried out on subcutaneous petroleum ether extract of *M. pumilum* in post-partum female wistar rats, hepatotoxic (sinusoidal degeneration of liver) and renotoxic (inflammation of renal tubules) effects were reported (Effendy *et al*., 2006). Subsequently, a 28-day toxicity study of the aqueous leaf extract of *M. pumilum* was conducted (Singh *et al*., 2009) in healthy male and female wistar rats. In this study, 50, 250, 500 and 1000 mg/kg daily oral doses of *M. pumilum* extract were administered to animals and clinical signs of toxicity and mortality were observed daily. Toxicity testing was carried out on samples of blood, brain, heart, lungs, stomach, small intestine, liver, spleen, kidneys and testes/ovaries. At the end of the study, no mortality or sign of toxicity and treatment-related changes, at all doses, were observed

in the *M. pumilum*-treated groups. However, at doses higher than 50 mg/kg, pathological changes in the histology of the liver, lungs and kidneys (in dose independent manner) were observed. In the liver, peri-portal inflammation was seen in a rat that received 250 mg/kg dose of *M. pumilum*, while mild bile duct hyperplasia was seen in three rats each in groups that received 500 mg/kg and 1000 mg/kg doses. Peribronchial lymphoid aggregates in lungs of rats were seen in all treatment groups and hypertrophy of muscular arteries with mild emphysema of alveoli was also observed in one of the rats that received 1000 mg/kg dose. One of the rats that received 250 mg/kg dose showed signs of necrosis and focal pyelitis in the kidneys. No pathological changes were seen in samples of brain, spleen, heart, blood, stomach, small intestine and ovary/ testes in all treatment groups.

In a reprotoxic study, the possibility of offspring being born with structural and functional defects as a result of *M. pumilum* supplementation was investigated using 2, 20, 200, 400 and 1000 mg/kg body weight oral doses (Ezumi *et al*., 2007) in pregnant Sprague–Dawley rats. Reproductive and teratogenic toxicity was observed and recorded as changes in maternal body weight (MBW), corrected maternal body weights (CMBW), gravid uterine weight, number of corpora lutea, number of implantation sites, percentage of foetal resorption, number of live foetuses, foetal weight and foetal sex ratio. Results obtained at the end of the study revealed that, at all doses, *M. pumilum* showed no evidence of congenital malformations in foetuses from all dams. Similarly, no significant agent-related effects on the MBW, gravid uterine weight, percentage foetal resorption, foetal weight and sex ratio, implantation sites and live foetuses were observed in animals of all groups. Also, no toxicity on reproduction, offspring survival rate, performance estrous cycle and post-natal growth was reported in this study.

4.2 Clinical trials

In a randomized double-blind study in postmenopausal Malay women, the effects of aqueous extract of *M. pumilum* at 280 mg/ day dose was compared with placebo (Azidah *et al*., 2012). Twenty-nine (29) menopausal women were treated with *M. pumilum*, while thirty-four (34) women were put on placebo. Signs of menopause were monitored and assessed before treatment (baseline) and at 6 months of treatment. Parameters such as blood pressure, fasting blood sugar, waist circumference, body mass index, lipid and hormonal profile were monitored after every two months of treatment. Results obtained showed significantly lower triglycerides levels in subjects receiving *M. pumilum* when compared to placebo. The values of other parameters were similar across the groups.

In another randomized double-blind pilot study, the safety and efficacy of 200 mg daily dosing of *M. pumilum* extract for 12 weeks on sexual health, lipid profile and pro-inflammatory markers of pre- and post-menopausal women were investigated (George *et al*., 2004). Plasma total cholesterol, lipoprotein (high and low density), triglycerides, antioxidants indicators, anti-inflammatory markers, hormones, liver function markers, blood hematology and chemistry, body weight and vital signs were monitored in the course of the study. Sexual health was assessed using scores on female sexual function index (FSFI) and short form-36 health survey (SF-36) tools. Results obtained showed no significant difference in both the FSFI and SF-36 scores between *M. pumilum* and placebo treatment groups. However, women in *M. pumilum* group recorded lower total cholesterol and urinary 8-isoprostane concentrations when compared to placebo group.

4.3 Drug-herb interaction potential

Cytochrome P450 complex (CYP450) is a family of monooxygenase enzymes that play key roles in metabolism and detoxification of drugs (George and Farrell, 1991). In humans, many families and subfamilies of CYP450 have been identified. Other than CYP3A4 subfamily, CYP2C subfamily is the second most abundant CYP450 making up 20% of total hepatic CYP and accounting for approximately 16 to 20% of the CYP-mediated biotransformation (Gray *et al*., 1995). Drugs substrates metabolized by this subfamily include warfarin, phenytoin, sulphonylureas, losartan, non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs), diazepam, mephenytoin, paclitaxel, amodiaquine, rosiglitazone, arachidonic acid and retinoic acid (Rettie and Jones, 2005).

In an *in vitro* study, the effects of aqueous, hexane, dichloromethane and ethanol fractions of *M. pumilum* on cytochrome P450 enzymes activities were investigated (Pan *et al*., 2012). Their performances were tested using high performance liquid chromatographic technique (HPLC). The inhibitory capacities of the extracts were measured as concentration that will cause 50% inhibition of enzyme activity and was expressed as IC_{50} and K_i (inhibition constants) values. Results obtained revealed that *M. pumilum* potently and differentially inhibited activities of CYP2C isoforms, but did not inhibit CYP3A4 isoforms significantly. The order in which CYP2C isoforms were inhibited was: dichloromethane > hexane > ethanol > aqueous. Thus, dichloromethane extract of *M. pumilum* notably showed strongest inhibition of CYP2C8 and CYP2C9 isoforms with a recorded K_i value below 1 mg/ml.

In another study carried out by Manda *et al*. (2014), the likelihood of *M. pumilum* to cause HDI *in vivo* was evaluated *in vitro*. In this study, the effects of its methanolic extract and 6 isolated compounds (resorcinol, belamcandol, demethylbelamcandaquinone B, Fathimol, primulanin, and ardisimamilloside H) from the roots of *M. pumilum* were tested on CYP450 enzyme complex (CYP2D6, CYP1A2, CYP3A4, CYPC9 and CYP2C19). Effects on P-glycoprotein (P-gp), a vital transporter protein that plays important role in drug metabolism by expelling toxins and drugs from cells as well as pregnane X receptor (PXR), a receptor that regulates expression of drug metabolizing enzymes were also studied. Results obtained also showed that methanolic extract from the roots of *M. pumilum* as well as its constituents strongly inhibited the most abundant of CYP450 subfamilies, CYP3A4, but only moderately and reversibly inhibited CYP2C9 and CYP2C19 sub-families, and minimally inhibited CYP2D6 and CYP1A2 subfamilies.

These results suggest that *M. pumilum* has a potential for drug-herb interactions with known substrate of CYP3A4 and CYP2C enzymes when taken concomitantly.

5. Conclusion

Research data on the pharmacological properties of *M. pumilum* reveals that it possesses osteoprotective activities in laboratory animals. The specific mechanism of action *via* which it exerts these effects, though not well understood, is proposed to be due to its estrogenic, anti-inflammatory and antioxidants properties. Toxicological studies have also revealed its relative safety in animals and humans. In view of these data, it can be concluded that *M. pumilum* is a potential safe alternative to estrogen in preventing and treating osteoporosis in postmenopausal women. However, in view of reports of phytochemical studies that revealed varying nature of phytochemicals (that are responsible for the proven biological activity) in different parts of the plant, further investigations are required in order to ascertain the actual plant part and possibly specific phytochemical compound(s) that could be responsible for the proven osteoprotective activities. Thus, further toxicological and pharmacological (including mechanistic) investigations should be conducted on extracts of the various plant parts and phytochemical fractions of this plant in order to obtain purer, safer and possibly more efficacious compound(s) with better understood mechanism of action that could serve as better candidate for future drug development.

Conflict of interest

We declare that we have no conflict of interest.

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