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PHARMACOGNOSTIC EVALUATION OF PELTOPHORUM DASYRRACHIS (MIQ.) KURZ BARK

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Abstract: This study is preliminary evaluation of pharmacognostic parameters of *Peltophorum dasyrrachis* (Miq.) KURZ bark. *P. dasyrrachis* belongs to Fabaceae family and is traditionally used in the treatment of diabetic ulcers. Three authentic samples that are regularly used for treatment were identified and collected by a folk healer, Mr. Kitti Nakhun, who has expertise in treating diabetic wounds using herbs in Ubon Ratchathani province. Macroscopic and microscopic characteristics of the crude drugs were illustrated. The plant part used was obtained from the dried brownish bark. Observation under the microscope found that this plant presented starch granules, calcium oxalate, prism crystal and reddish pigment. In addition, the physicochemical properties including foreign matter, loss on drying, total ash, acid-insoluble ash, water soluble extractive, ethanol soluble extractives were 0.96±0.09, 1.64 ± 0.56, 5.78 ± 0.20, 0.40 ± 0.31, 16.62 ± 1.07 and 22.20 ± 0.44 % w/w, respectively. Moreover, the high-performance liquid chromatography (HPLC) analysis exhibited a HPLC-profile and contained wound healing and improving substances; gallic acid and vanillin in a concentration range of 1.87 - 1.91 and 0.38 - 0.40 mg/g, respectively. This study provided scientific evidence for authentic identification, and quality control of *P. dasyrrachis* crude drugs.

Keywords: *Peltophorum dasyrrachis*, Pharmacognostic evaluation, HPLC-profile

INTRODUCTION

Peltophorum dasyrrachis (Miq.) KURZ is a tree reaching up to 30 meters tall widely found in Northeast Thailand and belongs to Fabaceae family. It is commonly known in Thai as "A Rang". Its bark is a polyphenol-rich part which exhibits several biological activities such as wound healing, antioxidative, anti-inflammatory and anti-bacterial effects (Mutabaruka *et al*., 2007; Chen *et al*., 2020). According to Traditional medicine as stated by Mr. Kitti Nakhun, the dried brownish bark of this plant has been used in diabetic ulcer healing recipes. Diabetic ulcers are a major cause of hospital admission and are important diseases that often lead to suffering, pain and poor quality of life for diabetic patients. The most problematic organ is the feet. Foot ulcer is a leading cause of lower-leg amputation (Snyder and Hanft, 2009). Currently, Mr. Kitti provides ongoing treatment to local people. Mr. Kitti has been successful in treating more than 80% of diabetic wounds, he is on a committee of folk healers of Ubon Ratchathani Province and has passed the selection criteria for the registration of folk healers with the Ministry of Public Health. In communities, a folk healer is viewed as an essential part of their culture. In the northeast Thailand, the majority of people respect folk healers and still use herbs for health care. Therefore, to realize the desired benefit from herbal preparations, an individual must take

the right plant and use it in the right way. Although it is generally believed that most herbal preparations are safe for consumption, some active substances could be toxic with undesirable side effects. The variety of the constituents due to genetic and environmental factors effect the standard of herbal medicines. Nowadays, the use of herbs in treating diabetic wounds is another very popular option. Studies have shown that external application of herbal medicine can activate local macrophages, has a chemotactic effect on leukocytes, enhances local immunity, regulates matrix metabolism, promotes local microcirculation, and has anti- inflammatory, antibacterial effects, etc. (Yang *et al*., 2015). Herbal medicine has been regarded as a cheap and green resource for wound healing chemicals. Among them, nature tannins in plant extracts have been reported effective in antibacterial (Petri *et al.*, 2014) and antioxidant activities (Maria *et al.*, 2015) and wound healing (Su *et al.*, 2017). Some tannins involved in these biological activities are gallic acid and vanillin. Gallic acid, part of hydrolysable tannins, is a potential antioxidant that directs the exposition of antioxidant genes. Gallic acid forced cell migration of keratinocytes and fibroblasts in both normal and hyperglycemic cases. Moreover, it activated factors known to be hallmarks of wound healing (Yang *et al.*, 2016). Vanillin has been recognized as an important bioactive compound presenting a range of relevant pharmacological activities, such as antimicrobial, antioxidant, antitumor and antifungal properties. The study of Tavares *et al.* (2018) showed that Chitosan membrane modified with new zinc (II)-vanillin complex is effective in chronic wound healing in diabetic rats.

Although, *P. dasyrrachis* bark is used in traditional Thai medicine for treating diabetic ulcers, its pharmacognostic specification and chemical substances have not been established. In this study, the stem bark of *P. dasyrrachis* from three authentic sources in Ubon Ratchathani province, Thailand were inspected following WHO guidelines of a quality control procedure for plant materials in order to determine the pharmacognostic parameters to guarantee quality. In addition, high performance liquid chromatography (HPLC) was used to determine the chemical components in this plant.

MATERIALS AND METHODS

Preparation of Plant Materials

Three samples of *P. dasyrrachis* were collected from different places in Ubon Ratchathani province, Thailand. All sets of crude drugs were authenticated by Kitti Nakhun (folk healer) and Jakrapong Thangthong (taxonomist from Faculty of Science, Ubon Ratchathani Rajabhat University). Voucher specimens (CTAM-157) were deposited at the Faculty of Thai Traditional and Alternative medicine, Ubon Ratchathani Rajabhat University, Thailand. All foreign matters of dried stem bark were removed. The clean crude drugs were pulverized for study. The chemicals were HPLC grade for HPLC analysis and were analytical grade for the other tests.

Examination of Macroscopic and Microscopic Characteristics

The pictures of *P. dasyrrachis* dried-crude drugs were taken with a camera and illustrated by proportional scale related to real size. The anatomical and histological profile of tissue cross sections and powder of *P. dasyrrachis* bark were studied under microscope to identify the structural features of the plant. Photographs were taken with a digital camera. The cell character was expressed as a line drawing (World Health Organization, 2011).

Determination of Physio-chemical properties

Loss on drying, total ash, acid insoluble ash, volatile oil content and extractive values of *P. dasyrrachis* bark were carried out according to "Quality Control Methods for Medicinal

Plant Materials" which was the guideline (World Health Organization, 2011). The quality control procedure for plant materials is concisely described below.

Three grams of powder samples were dried at 105°C and constantly weighed to determine loss on drying in percentage. Three grams of ground sample were incinerated at 500°C until completely ashing, then boiled the ash with 25 ml of HCl (70g/l; insoluble matter was incinerated and constantly weighed to obtain the percentage of acid – insoluble ash. Clevenger apparatus was used for volatile oil extraction. The determination of extractive matter value was carried out with 95% ethanol and water as solvents by using cold maceration method.

Determination of Chemical Component

High-performance liquid chromatography (HPLC) analysis was done according to the method previously described (Sripanidkulchai and Junlatat, 2014), with slight modification. Briefly, the retention times (RT) of each standard was compared to the sample. The samples were dissolved in methanol and sonicated for 15 min, then centrifuged with 12,000 rpm for 10 min. The HPLC system (Agilent Technologies 1260 Infinity) consisted of reverse phase C_{18} Agilent ZORBAX column (5 µm, 4.6 x 150 mm). An isocratic elution at 1.1 ml/min was performed with 1% Acetic acid in water: acetonitrile (93:7). The detection was carried out at 272 nm. The injection volume was 20 µL. Five commercial reference standards of phenolic compounds: gallic acid, vanillic acid, vanillin, coumaric acid, epicatechin gallate (1 mg/mL in methanol) were used to confirm their presence in the samples by identification through comparison with retention times. For the quantitative analysis of phenolic compounds, a calibration curve was obtained through injections of different concentration of the reference standards. The contents of phenolic compounds in the samples were calculated based on the corresponding peak areas and injected concentrations. Moreover, validation of quantitative HPLC method including precision and accuracy was determined.

RESULTS AND DISCUSSION

Pharmacognostic specification is a primary tool for identification, authentication and standardization of herbal medicines. From the analysis of 3 samples of the plant, the pharmacognostic specification of *P. dasyrrachis* was showed as follows.

Macroscopic and Microscopic Specification

Microscopic and macroscopic studies are the simplest and cheapest method to provide referential information for the herbal plant authentication. *Peltophorum dasyrrachis* (Miq.) KURZ is a tree which grows up to 30 meters high. The young shoots are brownish-red with fine hair, later glabrous. Leaves are bipartile with both halves pinnatifid or bipinnatifid, 15-40 cm long, stipules are rather large, 5-9 pairs of pinnae, 6-16 pairs of leaflets, sessile, oblong with emarginated apex; lower surface brownish pubescent, upper surface shining, glabrous, 5-10 x 10-25 mm. Racemes lateral 15-30 cm long with pubescent axis. Bracts ± persistent, linear with subulate tip, 7-9 mm long. Pedicels 20-40 mm. Petals yellow, hairy towards the base of upper side, 15-25 x 10-12 mm. Buds ovoid. Sepals brownish tomentose outside, 10 x 6 mm. Filament 10-15 mm; anther 4-5 mm. Ovary sessile, velutinous; ovules 4-8. Pods are flat, reddish-brown, 10-15 x 2-3.5 cm, tapering towards both ends; the wing-like margin 4-5 mm broad. Seeds transversely arranged, 4-8, flat, 10-12 x 5 mm (Larsen *et al*,1984). The outer bark of dry crude drug is brownish- black. Inner bark is reddish- brown sticky wood. The *P. dasyrrachis* branch and inflorescence drawing were exhibited in figure 1. The microscopic characteristic study found that the part used of this plant presented starch granules, calcium oxalate, prism crystal, reddish pigment, etc. as shown in figure 2-3.

Physicochemical Parameter

The physicochemical evaluation of *P. dasyrrachis* was established in table 1. Foreign matter, loss on drying, total ash, acid-insoluble ash, water soluble extractive, ethanol soluble extractives were 0.96±0.09, 1.64 ± 0.56, 5.78 ± 0.20, 0.40 ± 0.31, 16.62 ± 1.07 and 22.20 ± 0.44 % w/w, respectively. This powdered drug did not contain volatile oil constituent.

The physicochemical evaluation of herbal drugs was applied to control the quality of plant material and product (Arambewela and Aranwwawalan, 2010). The quality and purity of the powdered crude drug was determined by ash examination. The total ash value of *P. dasyrrachis* bark was found to be quite high. Acid insoluble ash value was low. The total ash value indicates the plant contained inorganic compositions such as phosphorus, alumina, magnesium and calcium oxalate crystals. *P. dasyrrachis* contained a high number of calcium oxalate crystals; the amount of substance remaining after acid treatment was quite low (Mammen *et al*., 2010). Loss on drying was used to determine the content of water and volatile matters in the crude drugs. Water content should be minimized for protection from chemical deterioration as well as enzymatic action enhancer and microbial adulteration (Mukherjee, 2007).

High Performance Liquid Chromatography Analysis

Gallic acid and vanillin were found in *P. dasyrrachis* bark extract. They are important substances which increase wound healing. Gallic acid (RT=2.542 min) and vanillin (RT=22.356 min) were in a concentration range of 1.87 - 1.91 and 0.38 - 0.40 mg/g, respectively. The HPLC chromatogram of *P. dasyrrachis* bark was shown in figure 4. These compounds found (gallic acid and vanillin) corresponded to the biological activity of this herb that is used to treat diabetic wounds (Yang *et al*., 2016; Tavares *et al.,* 2018). The HPLC method validation was performed in terms of precision and accuracy. For the precision, it was found that the corresponding relative standard deviation (%RSD) values were lower than 0.89% and 0.35% for gallic acid and vanillin, respectively. For accuracy, the recoveries were determined to be in the range of 97.82%-106.35% and 94.74%-105.98% for gallic acid and vanillin, respectively.

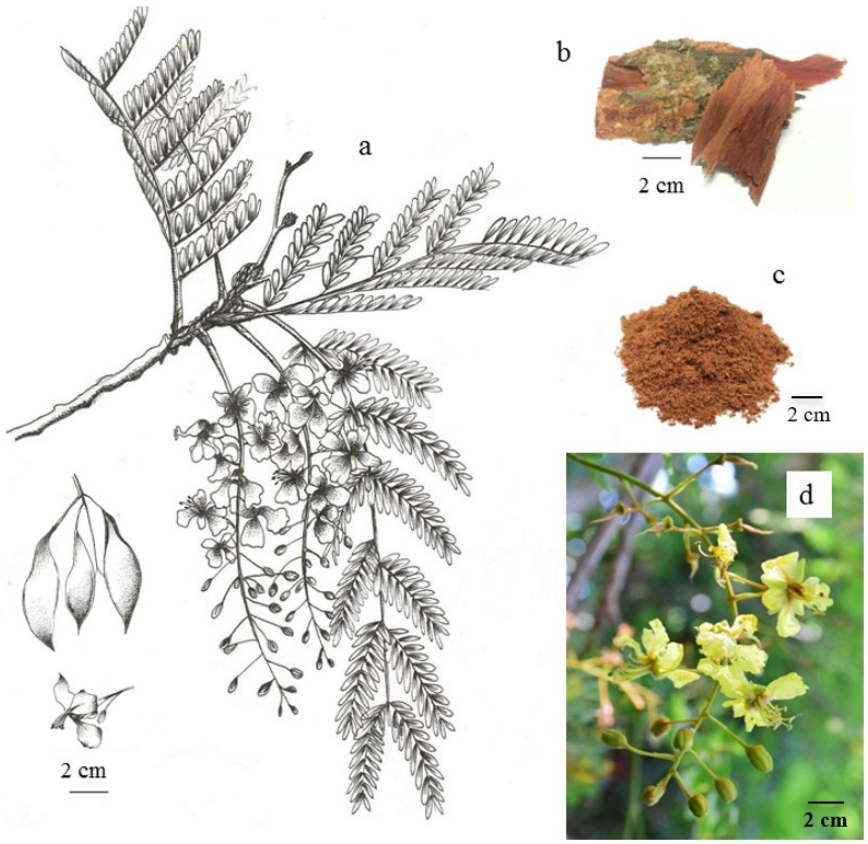

Figure 1. (a) branch, (b) crude drug, (c) powdered drug, and (d) inflorescence of *P. dasyrrachis*

Table 1. Physicochemical parameters of *P. dasyrrachis* bark

Parameter (% w/w)	Mean ± SD*
Loss on drying	1.64±0.56
Foreign matter	0.96±0.09
Total ash	5.78±0.20
Total acid-insoluble ash	0.40±0.31
Ethanol soluble extractive	22.20±0.44
Water soluble extractive	16.62±1.07
Volatile oil	ND

* Values were expressed as Mean±SD (each sample were done in triplicate)
ND=Not Detected

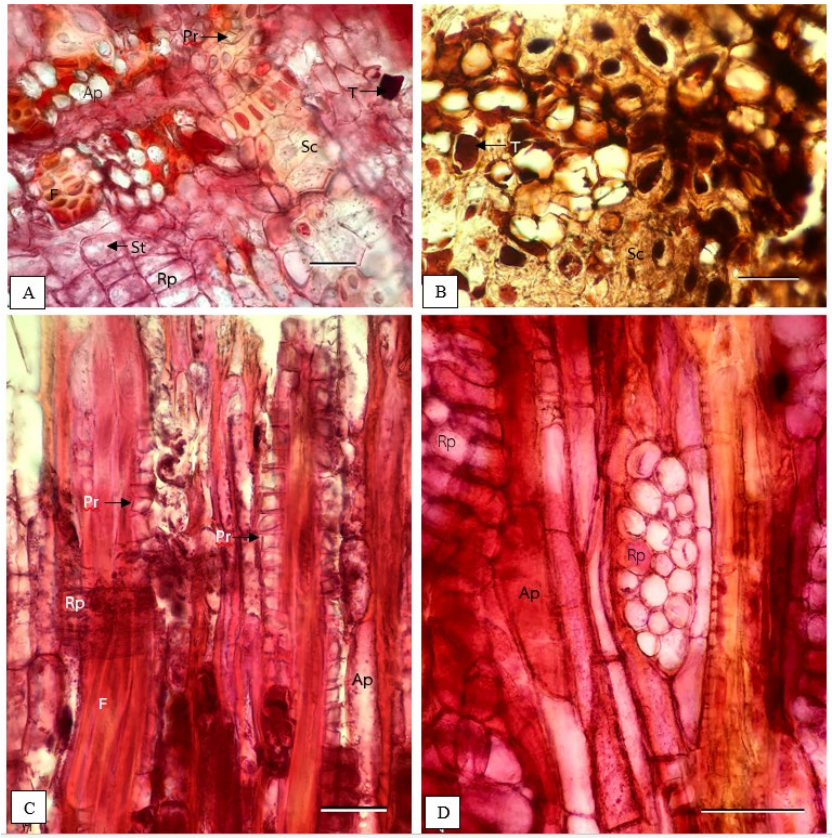

Figure 2. Anatomical character of *P. dasyrrachis*; (A) Transverse section of inner bark (B) Transverse section of outer bark (C) Radial section of bark (D) Tangential section of bark (scale 50 µm, Ap = Axial parenchyma cell, F = Fiber cell, Pr = Prism crystal, Rp = Ray parenchyma, Sc = Scleried cell, St = Starch grain, T = Reddish pigment)

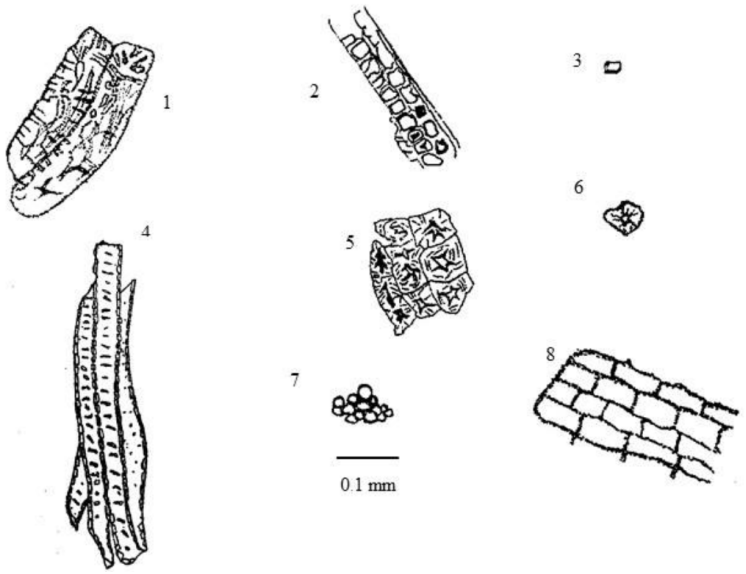

Figure 3. Powder of *P. dasyrrachis* bark; (1) sclereids (2) part of group of fibers with calcium oxalate prism sheath (3) calcium oxalate (4) fragment of fiber (5) part of group of sclereids (6) sclereid, transverse view (7) starch granules, and (8) cork in surface view

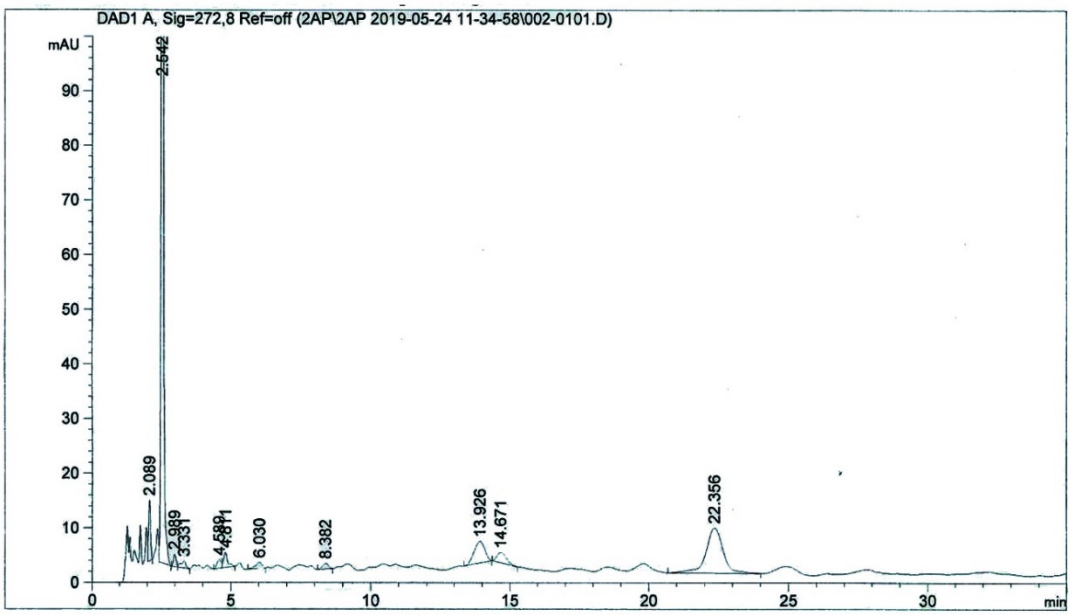

Figure 4. High-performance liquid chromatography chromatogram of *P. dasyrrachis* bark

CONCLUSION

Standardization of herbal crude drugs is important for herbal medicine quality control. This research, the pharmacognostic specification of *P. dasyrrachis* bark, was the first report. This study provides scientific information to support the use of herbs in treating diabetic wounds. For further study, there should be studies on the effectiveness of herbs and should have more samples in order to be classified according to international standards.

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REFERENCES

Arambewela LSR, Aranwwawalan L, 2010. Standardization of *Alpinia calcarata* Roscoe rhizomes. *Pharmacogn. Res.* 2(5): 285-8.

Chen G, He L, Zhang P, Zhang J, Mei X, Wang D, *et al*. Encapsulation of green tea polyphenol nanospheres in PVA/alginate hydrogel for promoting wound healing of diabetic rats by regulating PI3K/AKT pathway. *Mat. Sci. Eng. C*. 110: 110686.

Larsen K, Larsen SS and Vidal JE.1984. Leguminosae-Caesalpiniodeae In *Flora of Thailand* Smitinard T and Larsen K (Eds.) Vol. 4. Part 1. Bangkok: The Tistr Press; pp 1-132.

Mammen D, Daniel M, Sene RT. 2010. Variations in value of proximate analysis in *Aerva lanata* (L.), *Hedyotis corymbosa* (L.) and *Leptadenia reticulata* (Retz.). *J. Pharm. BioSci.* 1(4): 629-35.

Maria CF, Andrea Z, Pedro G, Maldonado A, Eric D, Celine PL. 2015. Grape seed and apple tannins: emulsifying and antioxidant properties. *Food Chem.* 178: 38-44.

Mukherjee PK. 2007. *Quality control of herbal drug: an approach to evaluation of botanical*. New Delhi: Business Horizons.

Mutabaruka R, Hairiah K, Cadisch G. 2007. Microbial degradation of hydrolysable and condensed tannin polyphenol-protein complexes in soils from different land-use histories. *Soil Biol. Biochem*. 39: 1479-92.

Petri W, Cruz Cristina D, Fletcher Graham C, Pajak Marta A, McGhie Tony K. 2014. Tannins and extracts of fruit byproducts: antibacterial activity against foodborne bacteria and antioxidant capacity. *J. Agric. Food Chem*. 62(46): 11146-56.

Snyder RJ, Hanft JR. 2009. Diabetic foot ulcers, effects on QOL, costs, and mortality and the role of standard wound care and advanced-care therapies. *Ostomy Wound Manag*. 55(11): 28-38.

Sripanidkulchai B, Junlatat J. 2014. Bioactivities of alcohol-based extracts of *Phyllanthus emblica* branches: antioxidation, antimelanogenesis and anti-inflammation. *J. Nat. Med.* 68: 615–22.

Su X, Liu X, Wang S, Li B, Pan T, Liu D, *et al*. Wound-healing promoting effect of total tannins from *Entada phaseoloides* (L.) Merr. in rats. *Burns*. 43: 830-838.

Tavares EA, Pontes TPA, Barbosa MM, Araújo AA, Araújo RF, Figueiredo JG, *et al*. 2018. Chitosan membrane modified with a new zinc (II)-vanillin complex improves skin wound healing in diabetic rats. *Front. Pharmacol*. 9: 1511.

World Health Organization. 2011. *Quality Control Methods for Medicinal Plant Meterials.* Geneva: WHO Press.

Yang DJ, Moh SH, Son DH, You S, Kinyua AW, Ko CM, *et al*. 2016. Gallic acid promotes wound healing in normal and hyperglucidic conditions. *Molecules.* 21(7): 899.

Yang Y, Wang F, Yin D, Fang Z, Huang L. 2015. Astragulus polysaccharide-loaded fibrous mats promote the restoration of microcirculation in/around skin wounds to accelerate wound healing in a diabetic rat model. *Colloids Surf. B: Biointerfaces*. 136: 111-8.