Application of negative pressure cavitation extraction (NPCE) on total phenolic compound extraction from *Millettia sericea* root

F Filianty

Department of Foods Technology, Faculty of Agriculture Industrial Technology, Padjadjaran University

Email: ffilianti31@yahoo.co.uk

Abstract. Production of total phenolic compounds from *Millettia sericea* roots was carried out using the NPCE method. This study aims to determine the effect of solvent concentration (60, 70, 80, 90%) and negative pressure (-0.4, -0.5, -0.6, -0.7 bar) in the NPCE system on *Millettia sericea* roots and investigate the increase in extraction yield compared to maceration methods. According to the results of the investigation, the best solvent concentration resulted from 70% methanol and -0.7 bar negative pressure. The results of the comparison of the NPCE extraction method with maceration exhibited a high escalate in extraction yield more than 3 times compare to maceration methods.

1. Introduction

Cavitation is a general fluid mechanics phenomenon which can take place whenever a liquid used in a machine generate pressure and velocity fluctuations in the fluid (e.g.: pumps, turbines, propellers) [1]. Cavitation can produce fluid energy that spreads locally in a very short duration and creates a strong area of energy dissipation. The method for producing cavitation consists of acoustic cavitation and hydrodynamic cavitation [2]. Ultrasonic cavitation is an acoustic cavitation method, which is proven to be an efficient method for extracting various secondary metabolites from plant material [3,4] and has been widely applied to the phytochemical fields [5–8]. However, ultrasonic cavitation has the disadvantage that thermosensitive compounds are easily degraded due to high-temperature increases during the process [9–11]. Therefore, cavitation techniques that do not cause an increase in temperature during the extraction of thermosensitive secondary metabolites were needed. Negative pressure cavitation employs the acoustic cavitation method in which cavitation occurs due to negative pressure conditions. This method was inexpensive and energy-efficient. During the process, there was no increase in temperature and relatively low temperatures due to nitrogen bubbles. The general mechanism of negative pressure cavitation extraction (NPCE) was that nitrogen flow continuously fed into the extraction system. Under negative pressure conditions, small nitrogen bubbles appear and rise between liquid-solid phases, resulting in the formation of highly unstable gas-liquid-solid. At that time there was a rapid mass transfer [12]. At the same time, nitrogen bubbles that burst due to cavitation cause corrosion on the surface of nearby solid particles. Corrosion of plant material causes cell wall degradation. As a result, the target compound which was bound to the cell wall of the material becomes exposed and the solvent can diffuse to dissolve the compound. As a result, the target compound is transferred from the matrix to the solvent [13].
The genus *Millettia* (family *Leguminoseae*, subfamily *Papilionoideae*) consists of more than 200 species that were native in the tropical and subtropical regions of Africa, Asia and Australia [14]. The plant *Millettia* (Class *Magnoliopsida*, Family *Fabaceae*) has a number of synonyms of which *Pongamia* and *Derris*. *Millettia sericea*, was one of *Millettia* species that are found in Indonesia, it was cultivated in provinces of West Java and locally called kawao. According to sugar farmer experiences, its roots were used as preservatives of *Arenga pinnata* juice. Where as the roots of this plant have not yet been phytochemically produced.

There have been several previous studies of biologically active substances of the *Millettia* species, alkaloids, flavonoids, tannins, triterpenoids, steroids and glycosides [15–20]. Previous studies have shown antimicrobials [21–22] and antioxidant activity [18]. Antimicrobial and antioxidant activity was influenced by phenolic compounds in *Millettia sericea* roots.

Production of total phenolic compounds in *Millettia sericea* roots can be done through an extraction process. Considering the effectiveness of the NPCE method, investigations on this material have never been carried out. Therefore in this study, engineering of total phenolic compounds extraction was carried out using the NPCE method. The factors that want to find out the effect were solvent concentration and negative pressure factors. To investigate the increased capacity of the extraction yield, the NPCE method was compared to the maceration method with different particle sizes. The concentration of the solvent will affect the ability to dissolve phenolic compounds. While the negative pressure will affect the level of cell wall damage in order to release phenolic compounds that will be dissolved in the solvent.

2. Materials and methods

2.1. Materials

*Millettia sericea* roots were collected from indigenous forests of Pangalengan, West Java, Indonesia and identified by Laboratory of Plant Taxonomy of The Biology Departement, Padjadjaran University, Indonesia. Voucher samples prepared and deposited in the herbarium for reference. The fresh roots was dried in the shade, powdered by a mill (Hammer Mill HMR-50 Type) and stored in a freezer for further studies.

Other materials used were chemicals for the extraction and analysis process. The chemicals used were analytical grade consisting of methanol, folin ciocalteu reagent, calcium carbonate and aquades. Nitrogen gas used was Ultra High Pure grade nitrogen with a 12 m³ tube capacity.

2.2. Extraction

Ten grams of *Millettia sericea* root powder was introduced to the NPCE device from the sample inlet portal. After the solvent (100 ml) was added, the device was connected to a vacuum pump. At the same time, a flow meter valve was opened and nitrogen was supplied to the device. The negative pressure of the device can be controlled by a valve. The extraction process was carried out under different conditions according to the design of the experiment. The effects of solvent concentration (methanol), negative pressure, and particle size on extraction yield (total phenolic content) were investigated.

2.3. Total phenolic content (TPC)

Total phenolic content of *Millettia sericea* root extract was determined using the method described by Basma et al [23] involving Folin-Ciocalteu reagent as oxidizing agent with slight modification. 200 μl plant extract (three replicates of 1.0 mg/mL) were introduced into test tubes; 500 μl of 10% Folin-Ciocalteu’s reagent, 500 μl of distilled water and 800 μl of 7.5% saturated aqueous sodium carbonate (Na₂CO₃) were added. The tubes were mixed thoroughly and allowed to stand in dark condition at ambient temperature for 30 min. Absorption was measured at 765 nm using spectrophotometer (Optima SP-3000, Tokyo, Japan).

Distilled water was used as a blank and gallic acid (0–125 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed as gallic acid equivalent per 100 gram of dry weight (mg GAE/100 g) of extracts. The phenolic contents of the sample were expressed as mg of...
gallic acid equivalent (GAE)/g of the extract, where GAE is the gallic acid equivalence (mg/mL) or concentration of gallic acid established from the calibration curve ($y = 0.0081x + 0.0421; r^2 = 0.989$).

2.4. Statistical analysis
All determinations were carried out in triplicates and data were expressed as mean ± Standard Deviation (SD). Statistical analysis was performed using One Way Analysis of Variance (ANOVA) followed by Tukey test and a $P < 0.05$ was regarded to be significant. Data were analyzed using the Minitab software.

3. Result and discussion
3.1. Mechanism of Negative Pressure Cavitation Extraction (NPCE)
The NPCE system consists of four areas: bubbles formation area, suspension area, axis air flow area and turbulent area [24]. In the bubbles formation area, the continuous of nitrogen flow into the extraction system. Under the action of negative pressure in the system, small nitrogen bubbles develop and ascend among the liquid-solid phase, results in the formation of the unstable gas-liquid-solid system. When the bubbles enter the suspension area, the volumes of bubbles change rapidly along with the outside pressure. Bubbles grow and collapse at certain flow regions with negative pressure. The bubbles grow and the continuous concave jet makes the surrounding liquid drops into liquid sheets which the liquid sheets with different solute concentrations can rapidly coalesce. Due to the mechanism, a mass transfer takes place among liquid drops [25].

On the other hand, the collapse of bubbles yielded cavitation and affected corrosion on the surface of solid particles. Thus the solvent can diffuse into the inside of solid particles. Subsequently, it causes intraparticle diffusion. In the axis air flow area, the compounds in the material were released into the solvent due to a profound collision and mass transfer effect between liquid drops and solid particles. In the turbulent phase, the process of mass transfer was accomplished by the action of the turbulent action effect. Thereby, the negative pressure cavitation of this system produces intensive cavitation-collision, turbulence, suspension and interface effects [24]. These effects integrated to form a mass transfer enhancing system and support the rapid transfer of target compounds from the matrix to the solvent and promote the extraction efficiency.

![Figure 1. Schematic diagram of Negative Pressure Cavitation Extraction (NPCE).](image-url)
3.2. Effect of solvent concentration
Based on the results of previous studies it was known that the best solvent for total phenolic extraction from the *Millettia sericea* roots was methanol solvent. To increase extraction efficiency, the best concentration of methanol was determined to allow higher total phenolics. Different concentrations of methanol will produce different polarities. Lower methanol concentrations produce a stronger solvent polarity, which supports the extraction of polar compounds. On the contrary, higher methanol concentrations are suitable for slightly polar compounds. In figure 2, it shows that at 70% methanol concentration resulted the highest total phenolic compound and decrease at higher solvent concentrations. This result shows that the polarity of phenolic compounds in *Millettia sericea* roots was slightly polar. In the application of sugar cane juice preservation, *Millettia sericea* root was even able to provide a good pharmacological effect in polar solvents [26]. In this case, the phenolic compound in the *Millettia sericea* root showed the optimum range of the methanol concentration would be between 70% to 80%. Therefore, it concentration was chosen for next NPCE process on the total phenolic extraction of *Millettia sericea* root.

![Figure 2](image1.png)  
**Figure 2.** Effect of solvent concentration on total phenolic content.

![Figure 3](image2.png)  
**Figure 3.** Effect of negative pressure on total phenolic content.

3.3. Effect of negative pressure
The formation and explosion of small bubbles under negative pressure conditions in the NPCE mechanism was the basic mechanism of this extraction method. Therefore, negative pressure was the main parameter that will affect the efficiency of the extraction results. Based on the results of this study exhibit that lower negative pressure causes the extraction results to increase. A decrease in negative pressure from 0.4 to 0.8 bar indicates an increase in the total phenolic content and reaches the highest yield at 0.8 bar which was 1.05 mg GAE/g (figure 3). This phenomenon explains that the reduced negative pressure that proceeds from an increase in the flow rate of nitrogen, causes an increase in the speed of formation of small bubbles that enough nitrogen bubbles are formed to form turbulent movements and small bubble break out. That condition then causes the optimal mass transfer.

3.4. Comparison NPCE with maceration methods
The NPCE method has been widely studied as a more effective extraction method than conventional methods. The NPCE method with the ability to degrade cell walls of materials can be compared with maceration methods which only rely on the ability of solvent diffusion. Based on the results of this study revealed that the NPCE method can increase total phenolic levels 3.67 times greater than the maceration
method. (figure 4). This value can be even higher at larger particle sizes. That was because the larger particle size has more material cell wall content. Within the cell wall, there are many phenolic compounds bound. Phenolic bound compounds will not be able to dissolve in extraction solvents because they are protected by cell walls. The cavitation process can degrade the cell walls of these materials and phenolics can be reached by solvents. The process causes an enhance in total phenolic transfer during extraction.

![Graph showing comparison extraction method based on particle sizes.](image)

**Figure 4.** Comparison extraction method based on particle sizes.

4. Conclusion
In this investigation, the NPCE process was applied to achieve the efficient extraction of total phenolic compounds from Millettia sericea roots. Solvent concentration and negative pressure factors influenced the extraction yield and NPCE comparison results with maceration methods show high efficiency. Based on the results of this study we conclude that NPCE represents a valuable alternative compared to maceration methods for the extraction of total phenolic compounds from Millettia sericea roots by considering optimal solvent concentration and negative pressure factors. The NPCE method was a sophisticated extraction method with high effectiveness and efficiency and also has the potential for industrial applications.

References
[1] Zhan D Y, Zhang S, Zu Y G, Fu Y J, Kong Y, Gao Y, Zhao J T and Efferth T 2010 Negative pressure cavitation extraction and antioxidant activity of genistein and genistin from the roots of pigeon pea *Cajanus cajan* (L.) Millsp.] Sep. Purif. Technol. 74 261–70
[2] Suslick K S, Gawienowski J J, Schubert P F and Wang H H 1983 Alkane sonochemistry J. Phys. Chem. 87 2299–301
[3] Xu J, Wu L, Chena W and Chang A C 2008 Simultaneous determination of pharmaceuticals, endocrine disrupting compounds and hormone in soils by gas chromatography–mass spectrometry J. Chromatogr. A 1202 189–95
[4] Zhang H F, Yang T S and Li Z Z 2008 Simultaneous extraction of epimedin A, B, C and icariin from *Herba Épimedi* by ultrasonic technique Ultrason. Sonochem. 15 376–85
[5] Li J, Momono T, Tayu Y and Fu Y 2008 Application of ultrasonic treating to degassing of metal ingots Mater. Lett. 62 4152–4
[6] Zhang H F, Yang X H, Zhao L D and Wang Y 2009 Ultrasonic-assisted extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism Innov. Food Sci. Emerg. 1 54–60
[7] Zhang Z S, Wang L J, Li D, Jiao S S, Chen X D and Mao Z H 2008 Ultrasound-assisted extraction of oil from flaxseed Sep. Purif. Technol. 62 192–8
[8] Shriwas A K and Gogate P R 2011 Ultrasonic degradation of methyl Parathion in aqueous solutions: intensification using additives and scale up aspects Sep. Purif. Technol. 79 1–7
[9] Desai V, Shenoy M A and Gogate P R 2008 Degradation of polypropylene using ultrasound-induced acoustic cavitation Chem. Eng. J. 140 483–7
[10] Mahamuni N N and Pandit B A 2006 Effect of additives on ultrasonic degradation of phenol Ultrason. Sonochem. 13 165–74
[11] Wang B, Wang Q, Liancai Z and Fengwei Y 2007 Degrade naphthalene using cells immobilized combining with low-intensity ultrasonic technique Colloid Surf. B 57 17–21
[12] Liu W, Fu Y J, Zu Y G, Kong Y, Zhang L, Zu B S and Efferth T 2009 Negative-pressure cavitation extraction for the determination of flavonoids in pigeon pea leaves by liquid chromatography–tandem mass spectrometry J. Chromatogr. A 1216 3841–50
[13] Li S M, Fu Y J, Zu Y G, Zu B S, Wang Y and Efferth T 2009 Determination of paclitaxel and its analogues in the needles of Taxus species by using negative pressure cavitation extraction followed by HPLC–MS–MS J. Sep. Sci. 32 3958–66
[14] Banzouzi J T, Prost A, Rajemiariiriraho M and Ongoka P 2008. Traditional Uses of The African Milletta Species (Fabaceae) Int. J. Bot. 4 406–20
[15] Simin K, Ali Z, Khaliq-a-Uz-Zaman S M and Ahmad V U 2002 Structure and biological activity of a new rotenoid from Pongamia pinnata Nat. Product Lett. 16 351–7
[16] Carcache-Blanco E J, Kang Y, Park E J, Su B, Kardono L B S, Riswan S, Fong H H S, Pezzuto J M and Kinghorn A D 2003 Constituents of the stem bark of Pongamia pinnata with the potential to induce quinine reductase J. Nat. Prod. 66 1197–202
[17] Kamau J W 2012. Phytochemistry and Biological Activity of The Root Extract Of Millettia Oblata. [Thesis] (Kenya: Jomo Kenyatta University)
[18] Hayyarimana L, Ndendoung S T, Tamokou J D, Atchadé A T and Tanyi J M 2012 Chemical constituents of Millettia barteri and their antimicrobial and antioxidant activities Pharm. Biol. 50(2) 141–6
[19] Singh R K, Nath G, Acharya S B and Goel R K 1997 Pharmacological action of pongamia pinnata roots in albino rats [A] Ind. J. Exp. Biol. 35 831–6
[20] Koysomboon S, van Altena E, Kato S and Chantrapromma K 2006 Antimycobacterial flavonoids from Derris indica Phytochemistry 67 1034–40
[21] More D R and Baig M M V 2013 Fungitotoxic properties of Pongamia pinnata (L) Pierre extracts against pathogenic fungi Int. J. Adv. Biotechnol. Res. 4(4) 560–7
[22] Sagwan S, Rao D V and Sharma R A 2012 In vivo and in vitro proportional antimicrobial activity in karanj (Pongamia pinnata): An Imperative Leguminous Tree IJRRPAS 2(6) 981–95
[23] Basma A A, Zakaria Z, Latha L Y and Sasidharan S 2011 Antioxidant activity and phytochemical screening of the methanol extracts of Euphorbia hirta L. APJTM 386–90
[24] Zhang D Y, Zhang S, Zu Y G, Fu Y J, Kong Y, Gao Y, Zhao J T and Efferth T 2010 Negative pressure cavitation extraction and antioxidant activity of genistein and genistin from the roots of pigeon pea [Cajanus cajan (L.) Millsp.] Sep. Purif. Technol 74 261–70
[25] Li S, Fu Y, Zu Y, Shi B, Wang Y and Efferth T 2009 Determination of paclitaxel and its analogues in the needles of Taxus species by using negative pressure cavitation extraction followed by HPLC–MS–MS J. Sep. Sci. 32 3958–66
[26] Filianty F, Raharja S and Suryadarma P 2010 Changes in the quality of sugarcane sap (Saccharum officinarum) during storage by adding the roots of Millettia sericea (Millettia sp.) and mangosteen (Garcinia mangostana L.) stem bark as preservatives J. Tek. Ind. Pert. 20(1) 57–64