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## Comparison of Extraction Techniques on Extraction of Gallic Acid From Stem Bark of *Jatropha curcas*

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**Abstract:** In this study, effectiveness of three different commonly applied extraction techniques for the determination of gallic acid in the stem bark of *Jatropha curcas* has been compared. The three different techniques that were used are namely as Soxhlet extraction, Ultrasonic-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE). Quantification of gallic acid in the extracts was done using HPLC. In general, all extraction techniques were capable of extracting gallic acid from the stem bark, but the recovery obtained using modern extraction techniques was higher than using conventional extraction techniques. In particular, MAE extracts presented a higher amount of gallic acid than Soxhlet and UAE extracts. Therefore, MAE was a simple and rapid technique that was useful for extraction of *Jatropha curcas* stem bark. In contrast, Soxhlet extraction only results in slightly ruptured cell pores, which could explain its low amount of gallic acid produced.

**Key words:** Gallic acid, Soxhlet, ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE), HPLC technique

### INTRODUCTION

Extraction and product recovery are the most imperative steps in evaluation of valuable biologically active compound from various plant parts. A desirable extraction technique should be simple, inexpensive, efficient, selective, environmentally friendly and compatible with various analytical techniques. However, limitation of the extraction process is usually due to the time constraint, labor intensive, lengthy operation techniques and highly cost of operations.

Classical extraction technique such as maceration and Soxhlet extraction are time consuming, require a huge amount of solvent and possible degradation of target compound due to overheating (Schmook and Serralta-Peraza, 1997). Modern extraction techniques, including Microwave-Assisted Extraction (MAE) and Ultrasonic-Assisted Extraction (UAE) are much faster and efficient.

Various parts of *Jatropha curcas* (Euphorbiaceae) had been used in many traditional medicines to cure many ailments. Gallic acid (3, 5, 7-trihydroxybenzoic acid) and its derivatives are the natural products of hydrolysis of tannin (Gubitz *et al.*, 1999). Significant biological activities of gallic acid such as an antioxidant (Martinez-Herrera *et al.*, 2006), anti-inflammatory (Kumar and Sharma, 2008), antifungal (Igbiosa *et al.*, 2009) and

carcinogenic properties (Gubitz *et al.*, 1999) have attracted considerable interest in pharmaceutical, food and agriculture industries (Mammela *et al.*, 2001).

The purpose of extracting phenolic compounds from plant materials is to release the compounds from the plant part where they are found by rupturing the plant tissues or by a diffusion process (Escribano-Bailon and Santos-Buelga, 2003). A high extraction efficiency that produces high extraction recovery is required in every extraction process. In order to increase extract recovery and preserve bioactivity, the extraction techniques need to be revised. Thus, the innovative extraction techniques such as UAE and MAE need to be explored and compared with the conventional extraction techniques.

The present study compared the ability of three extraction techniques namely as Soxhlet, UAE and MAE to extract gallic acid compound from the stem bark of *Jatropha curcas*. Four factors such as solvent composition, time, temperature and power had been studied for UAE and MAE. Each technique's performance was assessed by quantifying the amount of gallic acid using HPLC.

### MATERIALS AND METHODS

**Preparation of plant materials:** *Jatropha curcas* branches were collected from a plantation in Selangor,

Malaysia. The branches were cleaned by hand to remove foreign materials. Then, the bark was stripped from the branches and cut into small pieces prior to drying. An oven with a temperature of 60°C was used to dry the bark for 8 h. The dried bark was then grounded before it was sieved. A granulometric apparatus was used to obtain a homogenous particle size. Separation of the grounded sample was carried out with a sieve shaker (Fritsch) with various granulometric size sieves to obtain a homogeneous size of 1.0 mm. The samples were then kept in a seal plastic bag and store at room temperature.

**Chemicals and reagents:** Ethanol (with purity of 95% v/v) was purchased from R and M Chemicals Ltd. Acetonitrile and orthophosphoric acid both HPLC grades were purchased from Merck, USA. Gallic acid (HPLC grade) was purchased from Sigma-Aldrich, USA.

**Soxhlet extraction:** A Soxhlet apparatus was employed in which 10 g of sample was placed into a thimble with 300 mL of solvent composition (0, 20, 50, 70 and 95% ethanol) in a 500 mL round-bottom flask. Extraction was carried out for up to 6 h. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator.

**Ultrasonic-assisted extraction (UAE):** An ultrasonic bath (40 kHz, 230 Watt) was used in this study. The extraction of gallic acid was performed by adding 10 g of sample with 150 mL of solvent in a 250 mL of conical flask. The flask was then partially immersed into the ultrasonic bath. The bottom of the flask was approximately 5 cm from the bottom of the bath. Water in the ultrasonic bath was circulated and regulated to avoid temperature rising caused by exposure of ultrasonic. Four different factors were investigated namely as solvent composition, time, temperature and ultrasonic power. The extract was then filtered with filter paper (Whatman No. 1) and the filtrate was concentrated under vacuum at 50°C using a rotary evaporator. All the experiments were performed in triplicate.

**Microwave-assisted extraction (MAE):** MAE was carried out using domestic microwave (NN-S215 WF, 2450 MHz, Panasonic) with total capacity of 800 W. It was equipped with one 1000 mL closed quartz vessel, a temperature sensor, a temperature controller and a condenser. Ten grams of grounded *Jatropha curcas* stem bark was placed in quartz extraction vessel and 300 mL of solvent was added. Extraction process was carried out under different MAE conditions. The same factors as in UAE had also been studied in MAE. Then, each extract were filtered

through filter paper (Whatman no.1, USA) and concentrated under vacuum at 50°C using a rotary evaporator.

**HPLC analysis of gallic acid:** Gallic acid was determined by High-Performance Liquid Chromatography (HPLC). HPLC analysis was performed on an Agilent 1100 liquid chromatography system (Agilent Technologies, USA), equipped with vacuum degasser, four single solvent delivery pumps, a thermostated column compartment, a 20 µL sample loop manual injector and a diode-array detector. Samples were separated using Supelco Ascentis RP-Amide column (15×4.5 mm i.d., 5 µm particles size) at a temperature of 30°C. The mobile phase delivered at a flow rate of 1.0 mL min<sup>-1</sup> was a mixture of 85:15 (v/v) of 0.085% aqueous orthophosphoric acid and acetonitrile over 11 min. The diode-array detector was performed at 280 nm. The peak detected was identified by comparing their retention time with the standard. The concentration was calculated based on the calibration curve.

## RESULTS AND DISCUSSION

**Effect of solvent composition:** The effect of solvent compositions on the amount of gallic acid for three different extraction methods was shown in Fig. 1. All three extraction methods demonstrated that the improvement of extraction efficiency was observed with the addition of some amount of water. The amount of gallic acid increased as the solvent composition increased up until 50%. Usually, by varying the solvent polarity from water to ethanol, the extraction yield will increase. At the same time, product recovery will decrease with decreasing water percentage. This could be due to the relative polarity and the decrease in effective swelling of plant materials (Rouhani *et al.*, 2009). Alcoholic solvents have been commonly used as solvent to extract polyphenols from

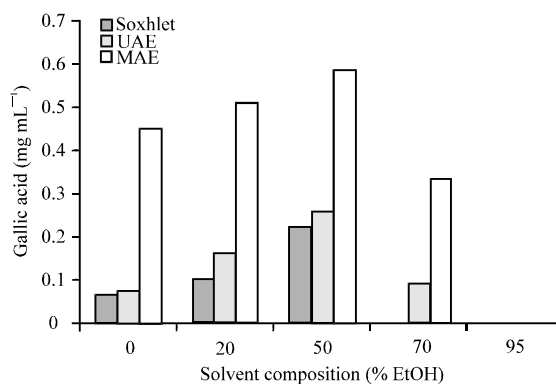


Fig. 1: Effect of solvent composition on the amount of gallic acid for Soxhlet, UAE and MAE

natural sources where they will give higher yield of total extract, even though they are not highly selective for phenols. Mixtures of alcohol and water have revealed to be more efficient in extracting phenolic constituents than compared to mono-component solvent system (Spigno *et al.*, 2007). Addition of water to organic solvents usually creates a more polar medium which facilitates the extractions of polyphenols (Spigno *et al.*, 2007).

Higher amount of gallic acid was observed when some outer forces were introduced. The outer forces involved were ultrasonic wave and electromagnetic radiation for UAE and MAE, respectively. In the case of UAE, a phenomenon called ultrasonic cavitation was produced in the solvent by the passage of an ultrasonic wave (Chen *et al.*, 2007). The intensity of ultrasonic cavitations in the solvent mixture was affected by the surface tension, viscosity and medium vapor pressure (Chen *et al.*, 2007). In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture was increased as the surface tension increased while the viscosity and vapor pressure decreased (Rostagno *et al.*, 2003). Water has a higher surface tension than ethanol, which needs higher energy to produce cavitation bubbles. Ultrasonication in low vapor pressure produces few cavitation bubbles that collapsed at a high intensity produces a shock wave that passes through the solvent enhancing mass transfer within the plant materials. Furthermore, solvent with lower viscosity has low density and high diffusivity, which can easily diffuse into the pores of the plant materials (Djilani *et al.*, 2006; Ou *et al.*, 1997; Mason *et al.*, 1996; Roldan-Gutierrez *et al.*, 2008).

For MAE, by addition of water, the mixture dielectric constant increase. This could help absorb microwave energy, thus increasing extraction efficiency. Although, the addition of water increases the dielectric constant, the dissipation factor will decrease. This means that although the solvent mixture could absorb high microwave energy as a result in increased dielectric constant, the mixture could not dissipate the heat effectively (Hemwimol *et al.*, 2006). As found in this investigation, solvent mixture with higher water content the extraction was low and not favorable.

In all the three methods used it shows the same pattern of results where 50% of ethanol concentration give the highest amount of gallic acid extracted. However, using MAE produced almost triple amount of gallic acid compared to Soxhlet and UAE. In this case, clearly 50% solvent composition was chosen to be the effective solvent composition and used to continue the study.

**Effect of time:** The time of extraction was evaluated in UAE and MAE techniques. The techniques used for the

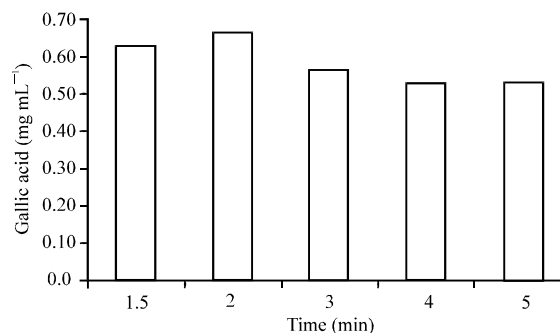


Fig. 2: Effect of time on amount of gallic acid for MAE

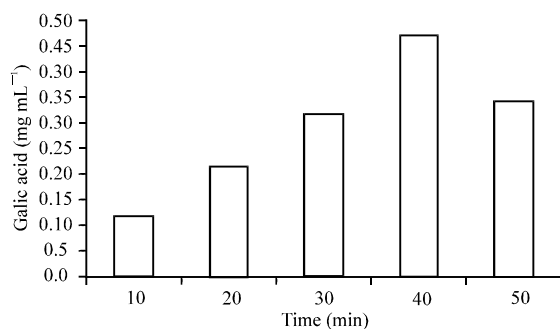


Fig. 3: Effect of time on the amount of gallic acid for UAE

isolation of gallic acid had a different effective extraction time. A comparison of the extraction time for the investigated techniques is shown in Fig. 2 and 3. The results showed that an increased in extraction time will increase the amount of gallic acid for MAE and UAE. MAE can reach the highest amount of gallic acid in 2 min while UAE needed 40 min. To some extent of time, the amount of gallic acid decreased when the time increased. This effect is related to the overexposure to the radiation or thermal degradation. Although, both techniques disrupt the plant cells, MAE extraction was more effective because it uses cell's internal water as the conductor medium for microwave. The extraction time is the shortest in case of MAE compared to UAE and Soxhlet. Soxhlet extraction is the most time-consuming technique where it needed 6 h of extraction time. From the results obtained, MAE is a rapid technique on the recovery of gallic acid.

**Effect of temperature:** The effect of temperature on the amount of gallic acid is shown in Fig. 4. It was found that the temperature from 35-55°C enhanced the product recovery. The amount of gallic acid will be decreased when the temperature was raised. Temperature affects many physicals properties, including viscosity, diffusivity, solubility and surface tension (Yang *et al.*, 2008). Additionally, increase in temperature will allow the solvent to have a higher capacity to solubilize analytes,

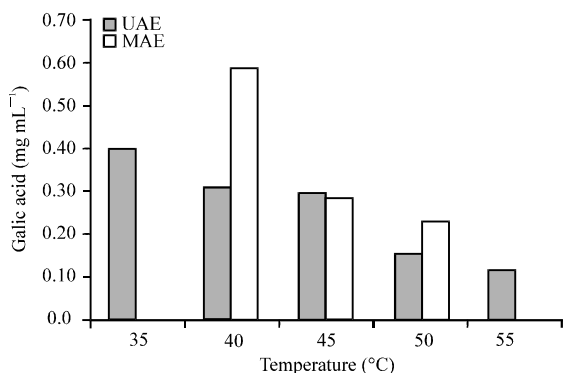


Fig. 4: Effect of temperature on the amount of gallic acid for UAE and MAE

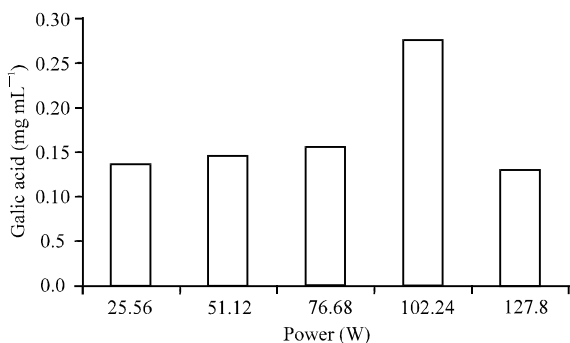


Fig. 5: Effect of power on the amount of gallic acid for UAE

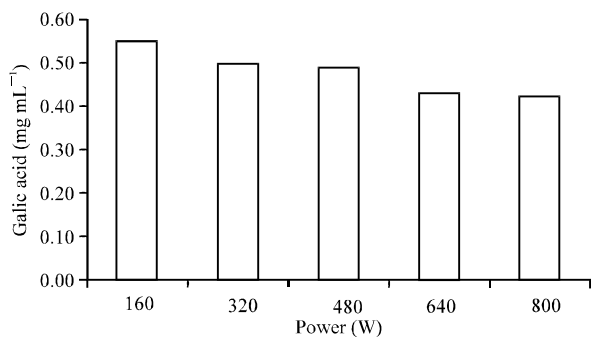


Fig. 6: Effect of power on the amount of gallic acid for MAE

while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively (Pan *et al.*, 2000; Camel, 2000). However, beyond certain temperature, phenolic compound can be denatured (Spigno *et al.*, 2007).

In all three extraction methods, extraction of gallic acid is taking place either in the range of studied temperature or at boiling point of solvent applied for the extraction. Higher recoveries were obtained using MAE than using Soxhlet extraction or UAE. There was no

amount of gallic acid for MAE at the temperature of 35°C might be because the temperature is too low for the microwave heating to heat up the water molecules of the plant cells. For UAE, the highest amount of gallic acid that was produced was at the temperature of 35°C while MAE was at 40°C.

**Effect of power:** The power for both UAE and MAE were evaluated and is shown in Fig. 5 and 6. From the results obtained, it was clear enough that there was an effect towards the amount of gallic acid when the power of these two equipments was studied.

It can be seen that there are improvements in the extracts obtained as ultrasonic output power increases from 25.56-102.24 W. An explanation for this is that the larger the amplitude of ultrasound wave travel through the mass medium, the more the cavitation bubbles were created and collapse (Hemwimol *et al.*, 2006). The violent shock wave and high speed jet might be generated, disrupting the cell walls. Solvent can penetrate into the cell and released the components from the cells into solvent. Meanwhile, the mass transfer rate was significantly enhanced. A decrease in the amount of gallic acid was obtained might be due to the compound that had been degraded, caused by excessive energy dissipation in the form of heat when high ultrasonic power was used. High amount of gallic acid was obtained when 102.24 W of ultrasonic power was used.

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. Microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted (Yan *et al.*, 2010). From this result, it can be noted that the use of microwave power can influence the percent yield. When microwave power was lower than 320 W, the extraction increased with the increase in microwave power. When microwave power was more than 320 W the extraction leveled out might be because all the gallic acid in the stem bark had been extracted at lower microwave power.

**Comparison of extraction methods:** The selection of extraction methods depends on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. Soxhlet extraction is the most common method of extraction. They are definitely user friendly but the

**Table 1: Comparison of extraction techniques on the amount of gallic acid**

Extraction method	Time	Temperature (°C)	EtOH (%)	Power (W)	Gallic acid (mg mL <sup>-1</sup> )
Soxhlet	6 h	80	50	NA	0.2232
UAE	40 min	35	50	102.24	0.4005
MAE	2 min	40	50	160	0.5477

NA: Not available

drawbacks are that they used a large amount of solvent and long time of extraction needed. Considering the massive use of solvent and long extraction time, this extraction method is not favorable to a commercial perspective.

UAE and MAE have received increasing attention as an alternative method. It has been used for several reasons: (1) reduced extraction time (2) reduced solvent usage and (3) improved extraction yield. By considering the economic and practical aspects, MAE is a strong novel extraction technique.

The efficiency of extraction using Soxhlet, UAE and MAE was compared and shown in Table 1. The findings demonstrate that UAE and MAE are promising extraction techniques that offer improved efficiency. From the comparison shows that at shorter time, MAE gave higher extraction yield compared to Soxhlet and UAE. The MAE can give the highest extraction selectivity for that extraction of gallic acid.

### CONCLUSION

Gallic acid degraded easily when they were exposed to high temperature, long period of time, high power for extraction and also affected by solvent composition. The amount of gallic acid was low when extracted using Soxhlet extraction technique. The effects of solvent composition, time, temperature and power had been investigated using UAE and MAE techniques. Comparisons of extraction techniques had been done towards the amount of gallic acid. MAE was the most efficient method where it provides high extraction efficiency in short time. Therefore, MAE is an alternative extraction technique for fast extraction of gallic acid from the stem bark of *Jatropha curcas*.

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### REFERENCES

Camel, V., 2000. Microwave assisted solvent extraction of environmental samples. *Trends Analytical Chem.*, 19: 229-248.

Chen, F., Y. Sun, G. Zhao, X. Liao, X. Hu, J. Wu and Z. Wang, 2007. Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography mass-spectrometry. *Ultrasonics Sonochem.*, 14: 767-778.

Djilani, A., B. Legseir, R. Soulimani, A. Dickob and C. Younos, 2006. New extraction technique for alkaloids. *J. Brazillian Chem. Soc.*, 17: 518-520.

Escribano-Bailon, M.T. and C. Santos-Buelga, 2003. Polyphenols Extraction from Foods. In: *Methods in Polyphenol Analysis*, Santos-Buelga, C. and G. Williamson (Eds.). Royal Society of Chemistry, Cambridge, ISBN-13:9780854045808, Pages: 383.

Gubitz, G.M., M. Mittelbach and M. Trabi, 1999. Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresour. Technol.*, 67: 73-82.

Hemwimol, S., P. Pavasant and A. Shotipruk, 2006. Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. *Ultrasonics Sonochem.*, 13: 543-548.

Igbinosa, O.O., E.O. Igbinosa and O.A. Aiyegoro, 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr. J. Pharm. Pharmacol.*, 3: 58-62.

Kumar, A. and S. Sharma, 2008. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. *Ind. Crops Prod.*, 28: 1-10.

Mammela, P., A. Tuomainen, H. Savolainen, J. Kangas, T. Vartiainen and L. Lindroos, 2001. Determination of gallic acid in wood dust as an indicator of oak content. *J. Environ. Monitor.*, 3: 509-511.

Martinez-Herrera, J., P. Siddhuraju, G. Francis, G. Davila-Ortiz and K. Becker, 2006. Chemical composition, toxic/antimetabolic constituents and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chem.*, 96: 80-89.

Mason, T.J., L. Paniwnyk and J.P. Lorimer, 1996. The uses of ultrasound in food technology. *Ultrasonics Sonochem.*, 3: 253-260.

Ou, Z.Q., L.Q. Jia, H.Y. Jin, T.H. Yediler and S.A. Kettrup, 1997. Ultrasonic detection and LC determination of linear alkylbenzene sulfonate in plant tissues. *J. Chromatography A.*, 44: 417-420.

Pan, X., H. Liu, G. Jia and Y.Y. Shu, 2000. Microwave-assisted extraction of glycyrrhizic acid from licorice root. *Biochem. Eng. J.*, 5: 173-177.

Roldan-Gutierrez, J.M., J. Ruiz-Jimenez and M.D.L. de Castro, 2008. Ultrasound-assisted dynamic extraction of valuable compounds from aromatic plants and flowers as compared with steam distillation and superheated liquid extraction. *Talanta*, 75: 1369-1375.

- Rostagno M.A., M. Palma and C.G. Barroso, 2003. Ultrasound-assisted extraction of soy isoflavones. *J. Chromatogr. A*, 1012: 119-128.
- Rouhani, S., N. Alizadeth, S. Salimi and T. Haji-Ghasemi, 2009. Ultrasonic-assisted extraction of natural pigments from rhizomes of *Curcuma longa* L. *Progress Color, Colorant Coatings*, 2: 103-113.
- Schmook, B. and L. Serralta-Peraza, 1997. *J. curcas*: Distribution and uses in the Yucatan Peninsula of Mexico. In: *Biofuels and Industrial Products from Jatropha Curcas*, Giibitz, G.M., M. Mittelbach and M. Trabi (Eds.). Dbv-Verlag fur die Technische University, Austria, pp: 53-57.
- Spigno, G., L. Tramelli and D.M. De Faveri, 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.*, 81: 200-208.
- Yan, M.M., W. Liu, Y.J. Fu, Y.G. Zu, C.Y. Chen and M. Luo, 2010. Optimisation of the microwave-assisted extraction process for four main astragalosides in *Radix Astragali*. *Food Chem.*, 119: 1663-1670.
- Yang, B., M. Zhao, J. Shi, N. Yang and Y. Jiang, 2008. Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chem.*, 106: 685-690.