The Potential of Modified Microwave Extraction System (MMES) to Extract Bioactive Components From Ferns

Faridah Kormin¹, Iqbal Ahmed¹*, Rosli bin Mohd Yunus¹,  
Zainal Abidin Mohd Yusof²

¹Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia  
²University Kuala Lumpur,  
Malaysian Institute of Chemical and Bioengineering Technology,  
78000 Alor Gajah, Malacca, MALAYSIA  
Email: zainalabidinn@micet.unikl.edu.my

Abstract—Extraction is an important step for separation of constituents from the plant material. The development of new separation techniques for the chemical, food and pharmaceutical industries has lately received a lot of attention. Compared with the traditional method of extraction using conventional heating, the microwave heating can be another alternative. Microwave heating technique is a simple, inexpensive and valuable tool in applied chemistry where lesser amount of solvent, simplified manipulation and higher purity of final product with lower cost. In this research, a novel modified microwave extraction system has been designed to extract bioactive component purpose. Dicranopteris linearis (Burm.) and Stenochleana palustris (Bedd.) are fern which is commonly used for traditional treatment. These ferns having medical values which is should not be overlooked by medicinal chemists and pharmacologists, either for modern treatment applications or for research purposes. Since today, not much consideration has been given towards the utility of these fern yet in modern medicinal treatment. Thus, the objective of this research is to propose a novel extraction device based on microwave technology which is quicker and allow substantial saving in energy and cost for the extraction of bioactive component from Dicranopteris linearis (Burm.) and Stenochleana palustris (Bedd.) for the next consideration.

Index Term—Microwave extraction, bioactive component, Dicranopteris linearis (Burm.), Stenochleana palustris (Bedd.).

I. INTRODUCTION

The development of new extraction technique from plant material has shown tremendous research interest and potential which lately received a lot attention. Traditional procedures include homogenization, filtration/centrifugation, steam-distillation, hydro-distillation, solvent and Soxhlet extraction and solvent-pressurize extraction (SPE) for the isolation and purification of chemical constituents from plant tissues present some disadvantages. Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and sometimes have lower efficiency. Moreover, many natural products are thermally unstable causing degradation of unsaturated or ester compounds through thermal or hydrolytic effects [1-3]. These shortcomings have led to considering the use of new techniques in the extraction of natural substances, which typically use less solvent and energy. Researchers in many universities are working on novel techniques that could lead to compact, safe, efficient, energy saving, and sustainable extraction processes [4-5] and, the bioactive compound obtained with microwave methods contained substantially higher amounts of oxygenated compounds and lower amounts of monoterpenes than conventional method. Compared with the traditional methods, microwave extraction has many advantages, such as reducing analysis time, simplified manipulation and work-up and higher purity of final product [6-8] because the microwaves heat the solvent or solvent mixture directly, and the direct interaction of microwaves with the free water molecules presents in the glands and vascular systems, which results in the subsequent rupture of the plant tissue and the release of the active compounds into the organic solvent. The microwave assisted extraction (MAE) is an interesting alternative to conventional methods, especially in the case of plant material extraction [9] and also offers new sample extraction techniques for matrices such as animal tissues, soils, water, consumer products, cosmetics, and others. The pressurized microwave-assisted extraction (PMAE) is one of the most often used systems which afford fast extraction with low solvent consumption [10]. Besides that the dynamic microwave-assisted extraction (DMAE) has also been found to be an efficient technique. Using a dynamic approach to extraction is generally advantaged, especially with respect to the partitioning of the solute into the extraction media. This can be highly efficient when fresh solvent is continuously introduced into the extraction vessel. The rate constant for desorption does not need to be large in comparison with the rate constant for adsorption for efficient removal of the target solute. A new concept of microwave hydro-diffusion and gravity (MHG) was introduced by Bousbia [11]. The extraction process
generate by combination of microwave heating and gravity working at atmospheric pressure presents an efficient extraction process, reduce waste, avoids water and solvent consumption and allows substantial energy savings.

The introduction of microwave has met the increasing demand for new extraction techniques, amenable to automation with reduced solvent consumption. In this context, microwave extraction appears as a promising method that still needs to be developed and studied in-depth, but which will probably be implemented in the near future for the analytical laboratory industrial, as a consequence of the significant and numerous advantages it shows over alternatives. Nevertheless, conventional techniques continue to dominate this application area. Some procedures have limited application whereas others are more broadly applicable. More research is needed to improve the understanding of extraction mechanism, remove technical barriers, improve the design and scale up of the novel extraction systems for their better industrial applications. Accordingly, a primary goal of our novel microwave extraction research is to develop of methods that are more efficient and yield pure and highly representative extracts.

II. MICROWAVE THEORY OF EXTRACTION

The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomena: ionic conduction and dipole rotation, which in most cases occurs simultaneously. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field. Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles [12]. As the field decreases, thermal disorder is restored which results in thermal energy being released.

Lately, microwave system has received a lot attention in the extraction of natural substances. An original method for extracting natural products by using microwave energy has been developed [13,14]. At 2450 MHz (which is the frequency used in commercial systems), the alignment of the molecules followed by their return to disorder occurs 4.9×10^9 times per second, which results in rapid heating. Unlike conventional heating which depends on conduction–convection phenomenon with eventually much of the heat energy being lost to the environment. This unique heating mechanism can significantly reduce the extraction time (usually less than 30 min) as compared to Soxhlet. During irradiation, the cells of sample matrix were thermally stressed, continuously raising the temperature of the cells and consequently, rupture of the cell walls and oil glands was made faster and the induction stage was eliminated [15]. Evidence has been presented that during the extraction of essential oils from plant material; microwave extraction allows the migration of the compounds out of the matrix. Indeed, microwaves interact selectively with the free water molecules present in the gland and vascular systems [16] this leads to localized heating, and the temperature increases rapidly near or above the boiling point of water. Thus, such systems undergo a dramatic expansion, with subsequent rupture of their walls, allowing the essential oil to flow towards the organic solvent. This process is quite different from classical solvent extraction, where the solvent diffuses into the matrix and extracts the components by solubilization. In addition, in microwave extraction a wider range of solvents could be used, as the technique should be less dependent on a high solvent affinity.

Microwave extraction process influenced by solvent nature and volume, extraction time microwave power, matrix characteristics and temperature [17]. Solvent choice for microwave extraction is dictated by the solubility of the target analyte, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent. Generally, by increasing the extraction time, the quantity of analyses extracted is increased, although there is the risk that degradation may occur. Microwave power and irradiation time are two such factors, which influences each other to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. The plant particle size and the status in which it is presented for microwave extraction can have a profound effect on the recoveries of the compounds. The particle sizes of the extracted materials are generally in the range of 100 μm - 2 mm. Fine powders can enhance the extraction by providing larger surface area, which provides better contact between the plant matrix and the solvent, also finer particles will allow improved or much deeper penetration of the microwave. Microwave power and temperature are very interrelated to each other and needs to be given special attention particularly when working with closed vessel system. In closed vessel systems, temperature may reach well above the boiling point of the solvent.

III. THE SIGNIFICANT OF STENOSCHLEANA PALUSTRIS AND DICRANOPTERIS LINEARIS IN THE CASE STUDY

Stenochleana palustris (Bedd.) is a fern widely distributed in Southern & North India through Malaysia to Polynesia and Australia. In Malaysia, young shoot of Stenochleana palustris, are eaten as vegetable are said to be particularly beneficial when eaten at breakfast-time during fasting month [18]. It’s also known as healthy food in Central Kalimantan and South Kalimantan where indigenous people in that place believe these vegetables good for health especially for source of Fe, and medicine for skin disease, malaria disease, fever, and ageless. Malaysian traditionally crushed the fern leaves and applied as a poultice to control fever. Peoples in Indochina used this plant to get rid of intestinal worms while New Guinea people treat boils, ulcers and wounds using this fern leaves. The fern rhizomes also beneficial for the treatment of
throat, gastric ulcers and used as a cooling agent and in the treatment of burns and ulcers [19].

*Dicranopteris linearis* (Burm.) possesses antinociceptive and anti-inflammatory activity and thus justify its traditional uses by the Malays to treat various ailments [20]. The leaves medically used as a poultice and infusion and decantation in cases of fever [18]. Research on this fern showed the significant antibacterial activities against Gram-positive strains [21]. Table 1 showed the ferns classification.

IV. EXPERIMENTAL PLAN DESIGN APPROACH
As microwave extraction is influenced by many factors as described and with these factors severely interacting with one another a statically optimization strategy needs to be adopted for determination of the optimum operating conditions.

| Table I |
|-----------------|-----------------|
| **CLASSIFICATION OF STENOCHLEANA PALUSTRIS (BEDD.) AND DICRANOPTERIS LINEARIS (BURM.)** |
| **Order**       | Blechnales       |
| **Family**      | Blechnaceae      |
| **Genus**       | Stenochlaena     |
| **Species**     | palustris        |
| **Local name**  | Paku miding, paku akar |
| **Solvent (%)** | Resam, Bengkawang |

Response surface methodology (RSM) which has recently attracted much the attention as an optimization technique in the field of numerical analysis. Furthermore it helps to obtain the surface contours that provide a good way for visualizing the parameter interaction [22]. The principle of RSM was described by Khuri and Cornell [23] and its objective is to optimize the response based on factors investigated [24]. Thus in this study are to propose a novel modified microwave extraction system for the extraction of bioactive component from ferns [*Dicranopteris linearis* (Burm.) and *Stenochleana palustris* (Bedd.)], at the same time create a green technology for the future scientific study of the extraction and introduce this advantages alternative in the analytical or production of biological compound in food, cosmetics and pharmaceutical industry. Moreover, an attempt is made to investigate the important factor considered of extraction performance using response surface methodology (RSM). RSM will use to identify the important interfacial reaction factor which influences the extraction performance [26], visualize the parameter interaction and also optimized microwave extraction system to obtain high yield of oil. The factors considered are the raw materials moisture, microwave power and extraction time where an extraction yield is a response variable. This investigation also highlights the various test results that confirm the validity and correction of the developed mathematical model for analyzing the effect of the various factors on the extraction volume.

Experiments will design on the basis of the experimental design technique that has been proposed by Box and Hunter [25]. The set of central composite design (CCD), comprises of $2^k$ full factorial design (+1, -1), where $k$ is the number of variables, superimposed by the centre point (+α, -α) and center runs (0) to improve the reliability of results and to reduce the size of experimentation without loss of accuracy. The star points allow estimation of the curvature in the model and establish new extremes for the low and high settings for all factors. The precise value of $\alpha$ depends on certain properties desired for the design point describes a circle circumscribes about the factorial square. A $2^k$ full factorial with central composite ($\alpha=2$) will used (in this case $k=3$) as shown in Table 2. The experimental design will generated using the Design Expert software (Version 6.0 Stat-Ease Inc.) which consist of factorial = $2^3$ = 8 corner points at +1 level, central composite = $2k = 4$ axial points at +2 level and center points at zero level repeated six times which involved 20 experimental observations as shown in Table 3.

| Table II |
|-----------------|-----------------|
| **VALUES OF THE VARIABLES AT FIVE LEVELS USED WITH THE DESIGN** |
| **Level** | **Solvent (%)** | **Microwave Power (Watt)** | **Time (min)** |
| -2 | 0 | 200 | 1 |
| -1 | 25 | 325 | 12 |
| 0 | 50 | 450 | 23 |
| +1 | 75 | 575 | 34 |
| +2 | 100 | 700 | 45 |

V. DATA ANALYSIS
Again Design Expert software (Version 6.0 Stat-Ease Inc.) will use to analyze the experimental data. Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i^2 + \sum \beta_{ij} x_i x_j$$

Where:

$\beta_0$ = are the regression coefficients for intercept,

$\beta_i$ = are the regression coefficients for linear,

$\beta_{ij}$ = are the regression coefficients for quadratic,

$\beta_{ij}$ = are the regression coefficients for interaction terms,

$x_i$ and $x_j$ = are the independent variables
TABLE III
EXPERIMENTAL DESIGN

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The Design Expert will use to generate response surfaces and contour plots while holding a variable constant in the second-order polynomial model. This model would likely be useful as an approximation to the true response surface. It is always necessary to examine the fitted model to ensure that it provides an adequate approximation to the true system and verify that none of the least square regression assumptions are violated. In this respect, the following test can be performed:

i) **Test for lack of fit**
As replicate measurements are available, a test for lack of fit on regression model can be performed. This test verifies the variation of the data around the fitted model. The non-significant for lack of fit indicates that there is no significant and systematic departure from the straight-line relationship, which has been fitted. The insignificant for lack of fit is achieved if the model fits the data well.

ii) **Test for residual analysis**
Residual analysis consists of terms to judge model adequacy [26]. It is used to estimate the difference between the observed and predicted response. In order to check the normality assumption, normal probability plots of the residual was constructed. A normality assumption is satisfied if the residual plots approximately along a straight line [27].

iii) **Test of significant based on F value.**
The $F$ ratio calculated from the data of the experiments explained the mean square of error between the regressions. The $F$-test and the corresponding $P$-value are used as a tool to check the significance of each of the coefficient which in turn are necessary so to understand the pattern of the mutual interactions between the best variables. The smaller the magnitude of $P$, the more significant is the corresponding coefficient [28]. In brief, a good model must be significant.

iv) **Test for $R^2$ and adjusted $R^2$**
In general, $R^2$ is used to decide whether a regression model is appropriate. It measures the amount of variation around the mean explained by the model. A value of $R^2$ close to 1 means a perfect fit to the experimental data. A value of $R^2 > 0.75$ indicates the aptness of the model. The $R^2$ decreases in range from 1 to 0 if the residual value increases. (Residual is the difference between the observed value and the fitted value). Adding the variables to the model increases $R^2$, regardless of whether the additional variable is statistically significant or not. For this reason, to obtain a more precise regression model judgment, an adj $R^2$ is preferably used. Adj $R^2$ measures the amount of variation in the dependent variables for which the model accounts. It adjusts the $R^2$ value based on the number of coefficient in the model. The adding of variables to the model does not always influence the adj $R^2$. If the value of $R^2$ and Adj $R^2$ differ dramatically, there is a good chance that the non-significant terms have been included in the model [27].

**Quantitative Determination**
Extracted oils obtained (extraction volume) will compare in term of quantitative determination. Yield of oil extracted will express as a percentage of the weight of oil obtained after extraction relative to the weight of dry sample used for extraction, as described hereinafter:

% oil content = weight of oil obtained after extraction × 100% weight of dry sample

**Screening for Antimicrobial Activity**
The paper disc diffusion method will employed to each sample of extracted oil from both ferns obtained during the CCD experiments and determine the antimicrobial activity. For these assays the cultures of the following microorganisms will used: one gram-positive (Staphylococcus aureus) and one gram-negative (Escherichia coli) bacteria and one yeast (Saccharomyces cerevisiae). All microorganisms will purchase from the Drug Prevention Bureau Ministry of Health Malaysia. Cultures of the microorganisms will maintain on nutrient agar (NA) medium. Briefly, a suspension of the tested microorganism (107-108 CFU/ml) will spread on the solid media plates. Filter paper discs of 6mm diameter (Whatman no. 1) will individually impregnated with 50ml of essential oil, then laid on the surface of the inoculated plates. At the end of incubation time (24h at 37ºC for bacteria, 48 h at 25ºC for yeasts), positive antibacterial and antifungal activities will
established by the presence of measurable zones of inhibition. The antimicrobial activity will record as the width (in millimeters, diameter of the disc included) of the zone of inhibition after incubation. Each test will perform in three replicates and repeated twice.

Screening for Antioxidant Activity
Each sample of extracted oil from both ferns obtained during the CCD experiments will analyze and compared. The extracted oil will evaluate for their activities to scavenge the stable DPPH radical using lutein as a positive control according to the method [29]. The affinity of the test material to quench the DPPH free radical was evaluated according to the equation: scavenging % = (A1 - A2) / A1 x 100%. A1 and A2 are absorbance at 517nm of the reaction mixture with sample and control, respectively. The IC50 values will obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

VI. RESULTS AND DISCUSSION
This study highlights the above, provides an overview of a novel technique of extraction, address the needs for validation of development of pharmacologically active extracts on plant and always stressing on safety, efficacy and quality of phyto-medications. Consequently in this study will expect to save and improve the positive aspects, reducing energy and production cost at aspect of 1:5. With this aim, the first important steps are to optimize the use of resources and minimize wastes and environmental impact. This requires the obtainment of new products, new synthetic pathways, new processes and new technologies, which are more environmentally kindly and address the Malaysian needs. Therefore, microwave system for the extraction of bioactive components is a logical consequence of the significant rate enhancement and higher product yield afforded by a modified novel microwave technology and the increase in productivity afforded by raw material chemistry.

Factors Effecting of Modified Microwave Extraction Parameters on Quantity of Extracted Yield
i) Solvent nature and volume
A correct choice of solvent is fundamental for obtaining an optimal extraction. Solvent choice for microwave extraction is dictated by the solubility of the target analyze, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent [30]. Particularly water has a high selectivity towards the analyze of interest excluding unwanted matrix components. This is important particularly in the extraction oil and biocomponents from epiphytes fern sample. Another important aspect is the compatibility of the extracting solvent with further chromatographic analytical steps. Microwave extraction can also be performed with the same solvent as used for the conventional extraction methods. However, the optimal extraction of solvents for microwave extraction cannot always be deduced from those used in conventional procedures. Dielectric properties of the water towards microwave heating play an important role in microwave extraction. The separation efficiency and selectivity of microwave extraction towards separation of targeting components (lignin, fats, oil, carbohydrates and amino acid) was investigated using single solvent extracting technique. It was established that both the efficiency and selectivity of microwave extraction depend significantly on the dielectric constant of the extracting water. Generally, in most cases water with good heating efficiency under microwave (low tan δ value) serves the purpose to its best. A water and fern ratio (1:1) mixture combination proved out to be a significant factor in the microwave extraction. One of such type of amount of water in the extracting solvent can penetrate easily into the cells of the plant matrix and facilitate better heating of the plant matrix. This in turn increases the mass transfer of the active constituents into the extracting solvent. 1:1 (v/v) was found to be the optimum extracting solvent composition for the extraction of bioactive component.

ii) Extraction Time
As in other extraction technique, time is another parameter whose influence needs to be taken into account. Generally, by increasing the extraction time, the quantity of analyzes extracted is increased, although there is the risk that degradation may occur. Often in this system 5-10 min is sufficient, but even 30 sec have been demonstrated to have given excellent recovery. Microwave extraction of oil extraction was found to increase up to 4 min and later decreased with the increase of time. In the extraction of components an overall high of 95% extraction was achieved with 10 min after which extraction yield dropped down, as over exposure may lead to thermal degradation of effective constituents [30]. But some extraction reports also reveal that varying extraction time does not significantly improves recovery [23, 33]. To determine the time needed to obtain complete recovery, extractions of samples of peppers were performed for different lengths of time. Extraction times of 2, 4, 6, 8 and 10 min were evaluated. Therefore, 5 min was selected as the extraction time, since this was sufficient to extract all the components present in fresh samples. A proper study on optimization of extraction time is vital because extraction time may vary with different plant part used. Irradiation time is also influenced by the dielectric properties of the solvent. Solvents like water, ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermo labile constituents.

iii) Microwave Power
Microwave power and irradiation time are two such factors, which influences each other to a great extent [33]. The temperature during microwave heating was difficult to control when the extractions occur at a temperature below the solvent boiling point [31]. A combination of low or moderate power with longer exposure may be a wise approach. In general, the extraction efficiency was improved by raising microwave power from 100 to 300 W in microwave extraction as shoed in
Fig. 1. During short extraction time (2 and 4 min), recovery was enhanced with increased microwave power. The difference of the components extracted between 100 and 300 W appeared to be more significant with short extraction time compared to long extraction time. High power with prolonged exposure always involves the risk of thermal degradation. Reports on the other hand also exist which shows that varying of power from 800 W to 1100 W had no significant effects on the yield of flavonoids extraction. At higher power level settings the extraction pattern was same whereas purity reduced substantially. Rapid rupture of cell wall takes place at higher temperature when kept at higher power, as a result together with the desired analyses impurities are also leached out into the solvent. Whereas at low power levels the cell wall rupture might take place gradually this enables selective microwave extraction.

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