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EXTRACTION OF ALKALOIDS FROM MICROWAVE DRIED *ADATHODA VASICA* LEAVES - A COMPARATIVE STUDY

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ABSTRACT

The effect of process parameters on the kinetics of microwave drying of *Adathoda vasica* leaves was studied. The variation of moisture ratio with time was tested with thin-layer empirical drying models. As Midilli's expression gave better predictions for all data points than other models, it was expanded further. The microwave dried samples were subjected to microwave-assisted soxhlet extraction and microwave-assisted extraction. Moreover, the process variables considered in the present study had a significant effect on the extraction yield of alkaloids from *A.vasica* leaves. Though a higher extract yield of 40.28 % was achieved for microwave-assisted soxhlet extraction under optimal conditions (microwave output power of 100 W, sample load of 10 g and solid to solvent ratio of 1:20 g/ml) in 10 hours, the microwave-assisted extraction consumed a maximum duration of 1178 sec for 100 W of microwave output power and yielded 37.83 % of extract.

KEYWORDS: ***Adathoda vasica, Drying, Empirical, Microwave-assisted, Alkaloids, Extraction yield***

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INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis[1]. From ancient times, plants have provided rich source of inspiration for novel drugs, as plant-derived drugs have made large contributions to human health[2]. Medicinal plants are of special importance taking into account their role in health protection and as a preventive or supportive therapy for numerous diseases and disorders[3]. *Adhatoda vasica,* a small evergreen shrub of the *Acanthaceae* family, is a well-known plant that has been used as a drug in the Indian systems of oriental medicine for over 2000 years[4]. The leaves of *A. vasica* are being used for the treatment of asthma, bleeding piles, breathlessness, cold, cough, chronic bronchitis, traumatic injuries, antispasmodic, rheumatic painful inflammatory swellings etc[5, 6]. The major alkaloids of the plant, vasicine and vasicinone in addition to deoxyvasicine, vasicol, adhatodinine and vasicinol were found to be biologically active and have been the subject of many chemical and pharmacological studies[7]. In particular, the combination of vasicine and vasicinone had shown pronounced bronchodialatory activity both in-vitro and in-vivo, comparable to theophylline[8]. Though extensive research has been carried out on herbs and medicinal plants, the reported literature on *A. vasica* has been restricted to develop reliable methods to isolate the major alkaloids present in the cell structures, either qualitatively or quantitatively, preventing their decomposition or chemical transformation[4, 9]. Although conventional analytical methods such as hydrodistillation, enfleurage, maceration, pressing and distillation are used for the extraction of bioactive compounds and essential oils from herbs and medicinal plants, the strict operating conditions, the long extraction period and the low extraction efficiency have made them undesirable to be used in practical applications. Further, these processes affect the quality of the final product (due to the loss of some volatile compounds) and degrade the unsaturated compounds (thermal effects) in the extract[10]. Newer extraction techniques like Enzyme-assisted extraction, Microwave-Assisted Extraction (MAE), Pressurized liquid extraction, Pulsed electric field extraction, Supercritical fluid extraction and Ultrasound assisted extraction have drawn the attention of researchers due to their feasibility, high safety and low cost. These processes are either selective or expensive and only few are mainly instrumental. Amongst all processes, MAE is proving to be a good option especially in research on extractions of medicinal plants[11, 12, 13].

Microwaves (MW) are electromagnetic waves (frequencies between 300 MHz and 300 GHz), made up of two perpendicular oscillating fields (electric and magnetic), which propagate through the material and the accompanying transport processes result in the dissipation of electric energy as heat[14]. Microwave heating is an excellent prospect to increase the rate of evaporation, where regions of higher moisture content within the material will absorb more microwave energy and heat generated throughout the material, leading to faster heating rates and shorter processing times compared to conventional heating, where heat is usually transferred from surface to the interior[15]. MAE combines rapid heating in the MW field with the traditional solvent extraction. The enhancement of this extraction process lies in the fact that either the solvent or the sample is rapidly heated by direct interaction with electromagnetic radiation. Further, the MW increases the pressure inside the material causing the cell structure to break, allowing the solvent to penetrate into the matrix[16]. It should be noted that this process however, involves the risk of overheating of the material when process parameters are out of control. Hence, the physical and thermal properties of these herbs should be determined for the ideal design of an extraction process to produce a product with high medicinal value. The study of *A. vasica* through MAE has thus necessitated a detailed understanding of the drying and extraction process parameters. As limited information is available on the effect of sample pretreatment on the extractability of a bioactive compound from plants, the effects of the process

parameters including MW output power, sample mass, MW irradiation time were investigated.

The present work is therefore, concerned with the following;

(i) to determine the effect of MW output power and sample amount on the MW drying kinetics of *A. vasica* leaves, towards the extraction of alkaloids; (ii) to develop a new empirical correlation for the parameters mentioned above by improvising upon a selected conventional drying model; (iii) to perform MAE and compare with Microwave-assisted Soxhlet Extraction (MSE), using different solvents and to evaluate the yield obtained from MW dried samples; and (iv) to characterize the preprocessed samples and the extract obtained.

METHODS AND MATERIALS

Materials

Fresh *A. vasica* leaves procured from the herbal farm of VIT University, Vellore, India, were washed thoroughly with distilled water and stored at 4°C, in a refrigerator. The specimen was authenticated by the Plant Biotechnology Division, VIT University, Vellore, India. At least ten measurements of the thickness of leaves were made at different points with a micrometer (Leica stage micrometer (MA285), Germany) and was found to be 0.5 mm. The initial moisture content, determined by vacuum oven method, was 70.72 % (w.b). All solvents and chemicals (hexane, acetone, ethanol) used for analysis were of analytical grade and purchased from SD Fine Chemicals Limited, Mumbai, India.

Drying equipment and experimental procedure

A conventional domestic MW oven (CE108MDF, Samsung Electronic Instrument Co. Ltd, India) having an inbuilt cavity of 358 x 327 x 231.5 (W x D x H in mm) with a maximum power output of 900 W and a frequency of 2450 MHz was modified for the MAE process. A hole of 16 mm diameter was drilled on top of the oven, through which a glass connector was fixed to interconnect the round bottom flask (250 mL) placed inside the oven cavity with the external condenser to condense the vaporized extract, which was then collected in a graduated cylinder. The hole was sealed tightly with proper MW leak-proof agents. Cold water (~ 4^{o} C) was used as condensing medium. A schematic of the experimental system is shown in Fig. 1. Experiments were performed at varied MW output power (100, 180 and 300 W) and sample mass (10, 20, 30, 40 and 50 g). The moisture losses of the samples were recorded at 30 sec intervals with an electronic balance, and the process was carried out until the weight of the sample remained constant. All measurements were carried out in triplicate.

MSE of Alkaloids from Adathoda vasica

The MW dried *A. vasica* leaves were powdered and 5 g of the sample was packed in a thimble of filter paper and then extracted for 10 hours with 98.5 % hexane (v/v) in a soxhlet apparatus at 55°C. The extract was then concentrated at 60°C under reduced pressure in a Buchi rotary evaporator and the dried powder was collected in Eppendorf tubes.

MAE of alkaloids from Adathoda vasica

MW dried leaves were ground with mortar and pestle and 5 g of the sample was transferred to the round-bottomed flask placed inside the MW oven. The solvent used was a mixture consisting of hexane, acetone and ethanol (2:1:1 (v/v)), with a boiling point of approximately $58^{\circ}C$[17]. The chosen solvent was added to the flask containing the ground leaves and then subjected to MW irradiation at different MW output powers (100, 180 and 300 W). The experiments were performed till maximum amount of solvent was evaporated. After MW heating, the mixture in the flask was allowed to cool down to room temperature and filtered with a Whatman No.1 filter paper.

Microwave extraction apparatus: schematic representation

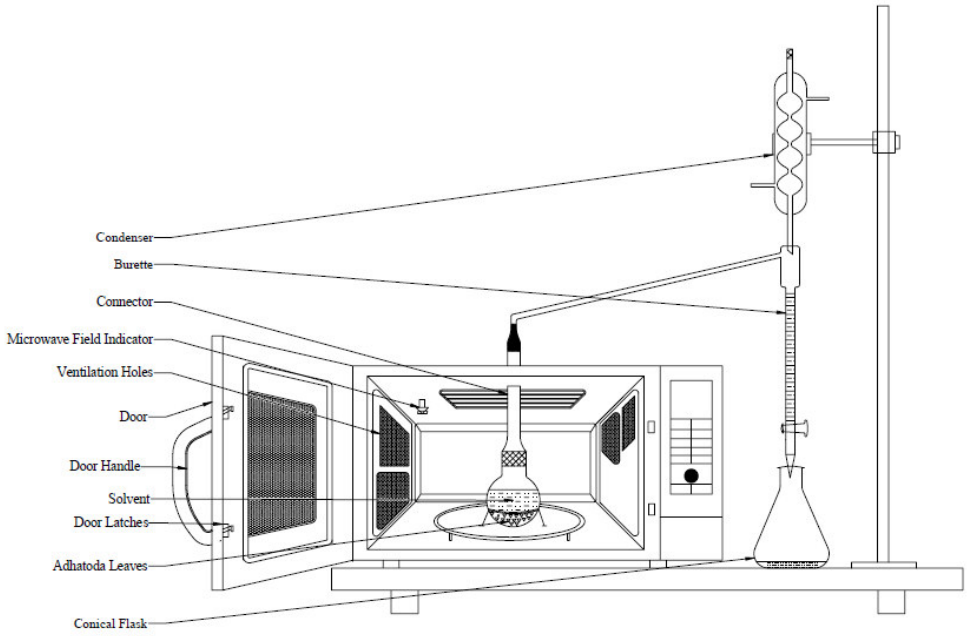

Figure 1
Microwave extraction apparatus: schematic representation.

The filtered extract was then concentrated at 60°C under reduced pressure in a Buchi rotary evaporator and the dried powder was collected in Eppendorf tubes. The percentage yield of alkaloids, obtained from *A. vasica*, by both methods was calculated using Eq. (1), as expressed by Prakash et al.,

[18] $$\text{Yield}\,(\%) = (W_c / W_o).100 \quad (1)$$

where, W_c and W_o are the weights of the crude extract (g) and the weight of dry powdered sample (g) respectively.

Microwave drying kinetics

The MW drying kinetics was evaluated on the basis of the mass lost by the *A. vasica* leaves. Mathematical differentiation of the drying kinetics allowed the determination of drying rates[19]. An analysis of the falling rate period was carried out to understand the drying kinetics by determining the effective moisture diffusivity (D_{eff}) and the influence of process variables on the effective moisture diffusivity. According to Fick's law the non-steady state diffusion, (assuming that the *Adathoda* leaves used can be approximated to a thin layer), is expressed by Eq. (2)

$$\text{MR} = (8/\pi^2)\exp(-\pi^2 D_{eff}/4L^2)t \quad (2)$$

where, D_{eff} is the moisture diffusivity (m^2/s^{-1}), L is half the thickness of the sample (0.00025 m), MR is the moisture ratio and t is the drying time (s). Linearising the above equation, the Fick's law becomes (Eq. 3):

$$\ln \text{MR} = \ln(8/\pi^2) - D_{eff}(\pi^2/4L^2)t \quad (3)$$

The following assumptions were made: (a) moisture is uniformly distributed throughout the sample, (b) mass transfer is symmetric with respect to the centre of the leaf, (c) surface moisture content instantaneously reaches equilibrium with air atmosphere; and (d) resistance to mass transfer at the surface is negligible compared to the internal resistance of the sample. The dependence of the effective diffusivity of the *A. vasica* samples on the MW output power and sample mass is represented by the following equation, (Eq. 4):

$$D_{eff} = D_o \exp(-E_a\, m/P) \quad (4)$$

where, E_a is the activation energy (W. g^{-1}), m is the sample mass (g) and P is the MW output power (W). The above equation is linearised and written as follows (Eq. 5):

$$\ln D_{eff} = \ln D_0 - E_a(m/P) \quad (5)$$

Data analysis and empirical modeling for MW drying

Study of drying kinetics and mathematical modeling of drying process is essential in order to design the suitable dryer[20]. The experimental moisture content data obtained for MW drying can be made dimensionless and simplified to MC_i / MC_o (MC_i is the moisture content at specific time, *'i'* and MC_o is the moisture content at initial time, 'o' (g water/g dry solids)). Eleven commonly used thin-layer drying models including Newton, Page, Henderson, Logarithmic, Wang and Singh, Diffusion, Verma, Two term exponential, Midilli, Modified Page and Two term equations (Eq. 6 – Eq. 16) were fitted to the drying curves (MR versus time), and the equation parameters were determined using non-linear regression analysis[30] (Table 1). All eleven models are derived into 'M^N' number of new models, where 'N' is the total number of constants and coefficients in the model and 'M' is the number of combination equations. A suitable empirical model to represent the effect of the MW output power on the constants and coefficients was investigated by using multiple combinations of different equations using Arrhenius type (a exp (-b/8.314 P), exponential type (a exp (b.P)) and linear type (ab.P) expressions in terms of MW output power[31]. The root mean square error (E_{RMS}), χ^2 and the modeling efficiency η_m were used as the primary criteria to select the best equation expressing the MW drying curves. The E_{RMS} gives the deviation between the predicted and experimental values. The lower the values of E_{RMS}, better the goodness of fit. The value for η_m also determines the suitability of the model equation and is required to approach unity for the best results.

Table 1

Values of the drying constants and coefficients of different models determined through regression method for Adathoda vasica.

Sl. No.	Model name and expression	Model constants	Statistical parameters		References	Eq.No.
1	Newton MR= exp(-kt)	k = 0.0088	RSS E_{RMS} χ^2 η_m	0.0030 0.0550 0.0031 0.9618	Motevali et al.(2010)	(6)
2	Page MR = exp (-kt^n)	k = 0.0110 n = 1.0000	RSS E_{RMS} χ^2 η_m	0.0071 0.0846 0.0076 0.9096	Sarimeseli (2011)	(7)
3	Henderson MR = a exp(-kt)	k = 0.0112 a = 1.0011	RSS E_{RMS} χ^2 η_m	0.0076 0.0877 0.0082 0.9029	Karathanos (1999) and Kaya et al.(2007)	(8)
4	Logarithmic; MR= a exp(-kt)+c	k = 0.0107 a = 1.0170 c = 0.0180	RSS E_{RMS} χ^2 η_m	0.0037 0.0611 0.0041 0.9528	Akpinar (2008)	(9)
5	Wang and Singh MR = 1 + at + bt^2	a = -0.0076 b = 1.46E-5	RSS E_{RMS} χ^2 η_m	0.0052 0.0724 0.0056 0.9338	Arslan and Ozcan (2010)	(10)
6	Diffusion MR= a exp(-kt)+(1-a) exp(-kbt)	k = 0.0110 a = 0.0999 b = 1.0000	RSS E_{RMS} χ^2 η_m	0.0071 0.0846 0.0079 0.9096	Wang et al. (2007)	(11)
7	Verma MR = a exp(-kt)+(1-a)exp(-gt)	k = 0.0061 a = 2.7141 g = 0.0049	RSS E_{RMS} χ^2 η_m	0.0026 0.0518 0.0029 0.9660	Karathanos (1999)	(12)
8	Two term exponential MR= a exp(-kt)+(1-a) exp(-kat)	k = 0.0105 a = 1.6502	RSS E_{RMS} χ^2 η_m	0.0026 0.0512 0.0028 0.9669	Midilli and Kucuk (2003)	(13)
9	Midilli MR = a exp(-k[t^n]+bt)	k = 0.0003 a = 0.9717 n = 1.6993 b = 0.0004	RSS E_{RMS} χ^2 η_m	$1.0x10^{-4}$ 0.0101 $1.2x10^{-4}$ 0.9837	Midilli et al. (2002)	(14)
10	Modified Page MR= exp(-kt)^n)	k = 0.0101 n = 1.0000	RSS E_{RMS} χ^2 η_m	0.0046 0.0680 0.0049 0.9415	Wang et al. (2007)	(15)
11	Two term MR= a exp(-k_1 t)+ b exp(-k_2 t)	k_1 = 0.0091 k_2 = 0.0088 a = 5.0091 b =-3.9150	RSS E_{RMS} χ^2 η_m	0.0019 0.0439 0.0022 0.9756	Wang et al. (2007)	(16)

Characterization

Scanning Electron Microscopy (SEM) was used to magnify and visualize the micro-structure of the dried *A. vasica* leaves. SEM processing of the fractured surfaces was performed using FEI Quanta FEG200 High Resolution SEM, Japan. Fourier Transform Infra Red (FTIR) spectra of *A. vasica* were obtained to gain knowledge about the distribution of the functional groups in the extract. Perkin Elmer (model RX1) spectrometer was used at a scan rate of minimum 20 cycles.

RESULTS AND DISCUSSION

Drying kinetics

To investigate the effect of MW output power and sample mass on moisture content, moisture ratio, drying rate and drying time, three MW output powers were used for drying five different sample mass. The time required to dry *A. vasica* samples from an initial moisture content of 70.72 ± 1 % (w.b.) to the final moisture content of 12.1 ± 1% (w.b.) was found to be 58, 95 and 590 sec at 300, 180 and 100 W respectively (Fig. 2a). The drying time obtained in this study was inversely proportional to the MW output power levels applied. There was no significant difference (35.9 %) in the drying time, when the MW output power was reduced from 300 to 180 W. However, while the MW power was reduced from 180 to 100 W, the drying time was significantly increased (84.66 %). It was found that 100 W is an optimum wattage for drying of *A.vasica* leaves as there wasn't any significant change in the microstructure. Moreover at 100 W, the essential bioactive components present in the leaf were unaffected. The entire drying process was restricted to a falling-rate drying, and a lack of constant rate drying period was also observed (Fig. 2a). This may be due to the fact that, higher moisture content in the sample would have contributed to an effortless moisture liberation, whereas it was difficult to remove water at a later stage, as equilibrium moisture content was approached. The mass of the sample plays a major role in arriving at the desired final moisture content, which explains the drying behavior of the samples. As seen from Fig. 2b, for a constant MW output power of 300W, increasing the sample mass from 10 to 20 g increased the total drying time from 120 sec to 140 sec (an increase by 1.16 fold). Similarly, a further increase in the mass to 30, 40 and 50 g., increased the total drying time to about 170, 210 and 220 sec, which indicated an increase by 1.42 fold, 1.75 fold and 1.83 fold respectively. It was observed that irrespective of the MW output power used to dry the sample, the drying rate was higher for the least mass (10 g) followed by 20, 30, 40 and 50 g. Further, the experimental results illustrated that during MW drying of *A. vasica* leaves, there was a lack of constant rate period and drying took place only during the falling period in which internal diffusion is the control mechanism for all the sample quantities dried (10 to 50 g). Similar trend was observed by Ozbek and Dadali[32] for microwave treatment of mint leaves.

Moisture content versus drying time of Adathoda vasica (a) at various microwave output powers for sample mass of 10 g, (♦ 100 W; □ 180 W; ▲ 300 W).

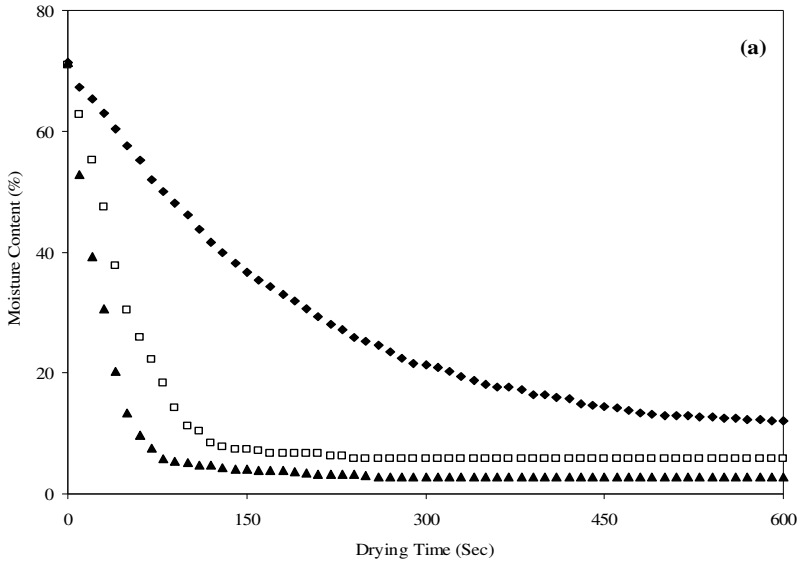

Figure 2(a)
Moisture content versus drying time of Adathoda vasica at various microwave output powers for sample mass of 10 g, (♦ 100 W; □ 180 W; ▲ 300 W).

Moisture content versus drying time of Adathoda vasica at various sample mass for microwave output powers of 300 W, (▲ 10 g; ◊ 20g; ♦ 30 g; □ 40 g; ■ 50 g).

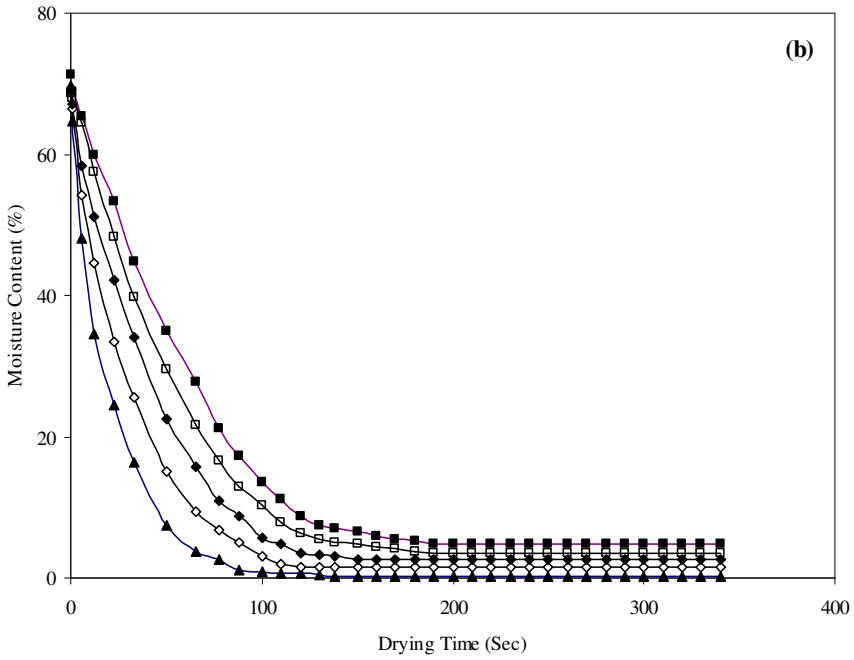

Figure 2(b)
Moisture content versus drying time of Adathoda vasica at various sample mass for microwave output powers of 300 W, (▲ 10 g; ◊ 20g; ♦ 30 g; □ 40 g; ■ 50 g).

Empirical modelling of drying curves

The drying kinetics data obtained were fitted to the eleven existing drying models, and the regression analysis was performed on the drying variables (MW output power, sample amount and drying time). Among all, the Midilli et al.,[29] model (with drying rate as a log–log and linear function of time) was found to be the most adequate in describing the MW drying process, since it gave the best fit for all the experimental data points owing to the lowest values of E_{RMS} (0.0101) and χ^2 (1.2 x 10^{-4}) as well as the highest value of modeling efficiency η_m (0.9837) (Table 1). Hence, Midilli's expressions were considered for further studies. As stated earlier eighty one new expressions were developed from Midilli's model using M^N combinations. Among these, Eq. (17) gave the best fit.

$$MR = \left(a.\exp\left(-a_1/8.314P\right)\right)\exp\left(-k.\exp\left(k_1.P\right)\right)\left(t^{n.\exp\left(-n_1/8.314\right)}\right) + \left(b.\exp\left(-b_1/8.314P\right)\right)t \quad (17)$$

The statistical parameters of the chosen equation were found to have least E_{RMS} and χ^2 value of 0.00533 and 3.19 x 10^{-5} respectively with a modeling efficiency η_m of 0.9993, for the model constants 'a' (1.0375); 'a_1' (0.9997), 'k' (0.9996); 'k_1' (-0.050); 'n' (0.9359); 'n_1' (8.3 x 10^{-5}); 'b' (0.0002) and 'b_1' (0.00023) (Eq. 18)

$$MR = \left(1.0375.\exp\left(-0.9997/8.314P\right)\right)\exp\left(-0.9996.\exp\left(-0.0501P\right)\right) . \left(t^{0.9359.\exp\left(-8.3x10^{-5}/8.314\right)}\right) + \left(0.0002.\exp\left(-0.00023/8.314P\right)\right)t \quad (18)$$

Further, the reliability of the established model was evaluated by comparing the predicted moisture ratio under any particular drying conditions with the experimental moisture ratio (Fig. 3). The predicted data banded over the straight line of the 1:1 ratio, with a value for the determination coefficient (R^2) of 0.9995. However, it should be noted that the data obtained is specific to the MW dryer used and can successfully be used to estimate the moisture content of *A. vasica* at any time during the MW drying at MW output power of 100 W.

Estimation of effective diffusivity and activation energy

The moisture diffusivity determined by the method of slopes varied from 0.279 x 10^{10} to 4.285 x 10^{10} m^2/s for all samples. The linear relationship between ln(MR) and drying time (t) for the MW output power of 300W (for a constant sample mass of 20 g) resulted in a higher R^2 value of 0.9919, while the least sample mass of 10 g (at constant MW output power of 100W) produced a higher R^2 of 0.9939. A similar trend was noted for other MW output power and sample mass (Table 2). At lower MW output power and higher sample load, a slight deviation from the linearity was observed; this may be attributed to more shrinkage of the product, non-uniform distribution of moisture during drying, and variation in effective moisture diffusivity with moisture content[33]. It was observed that diffusivity was directly proportional to MW output power and inversely proportional to the sample mass. It may be assumed that higher MW power and lower sample size have helped in increasing the energy transferred to the material which would have raised the vapor pressure inside the leaves, leading to greater moisture diffusivity[34]. But, the diffusivity values were inconsistent with those results existing in the literature for other leaves including mint, spinach, tea, dill and parsley[35, 36].

Comparison between predicted and experimental moisture ratio of Adathoda vasica for the derived Midilli model (Eq.17)

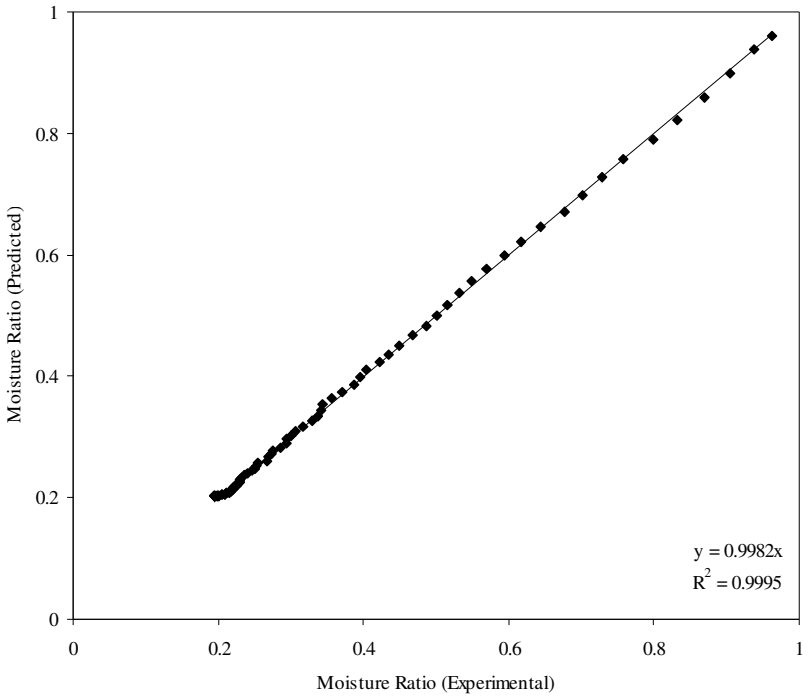

Figure 3
Comparison between predicted and experimental moisture ratio of Adathoda vasica for the derived Midilli model (Eq.17)

Table 2
Estimated drying indices of the best empirical drying model at various microwave output power and sample mass.

Sample Mass, g	MW output power, W	Diffusivity Coeffficient, $(D_{eff})_{th}.10^{10}$, $m^2.s^{-1}$	Activation Energy (E_a), $W.g^{-1}$		k_{th}, min^{-1}
			$\ln .D_{eff} = \ln D_0 - E_a\left(\frac{m}{P}\right)$	$\ln .k = \ln k_0 - E_a\left(\frac{m}{P}\right)$	
10	100	0.768	2.556	2.502	0.170
20		0.595			0.132
30		0.461			0.103
40		0.357			0.080
50		0.276			0.062
10	180	2.777	3.1932	3.298	0.173
20		2.325			0.144
30		1.947			0.120
40		1.631			0.100
50		1.365			0.083
10	300	4.338	4.813	4.842	0.177
20		3.695			0.150
30		3.147			0.128
40		2.680			0.109
50		2.283			0.092

The activation energy calculated from Eq. (5) (by plotting the natural logarithm of D_{eff} versus ratio of sample mass to MW output power (m/P)), was found to be a straight line for all ranges of MW output power and sample mass studied, indicating Arrhenius dependence. For the prediction of the relationship between drying rate constant and effective moisture diffusivity, an exponential expression based on Arrhenius equation (Eq. 19) performed for Okra was used[32].

$$k = k_0 \exp(-E_a\, m/P) \quad (19)$$

where, 'k' is the drying rate constant (min^{-1}) and 'k_o' is the pre-exponential constant (min^{-1}). The above equation could be converted to a straight line form as in Eq. (20).

$$\ln k = \ln k_0 - E_a(m/P) \quad (20)$$

Since Eq. (17) described the effect of drying variables better, the drying rate constant 'k' obtained from it was used. The values of activation energy obtained from Eq. (5) and Eq. (20) were found to be similar. The theoretical values of drying rate constant (k_{th}) obtained from Eq. (19) and the theoretical values of effective moisture diffusivity $(D_{eff})_{th}$ obtained from Eq. (4) were found to fit sufficiently to Eq. (21), with R^2 value of 0.9997. The value of constant (α) was obtained as 0.223 x 10^{10} min^{-1} m^{-2} s. The fitness of the data with the Eq. (21) is illustrated in Fig. 4.

$$k_{th} = \alpha.(D_{eff})_{th} \quad (21)$$

Relationship between drying rate constant, k_{th} and effective moisture diffusivity $(D_{eff})_{th}$ for microwave drying of Adhatoda vasica.

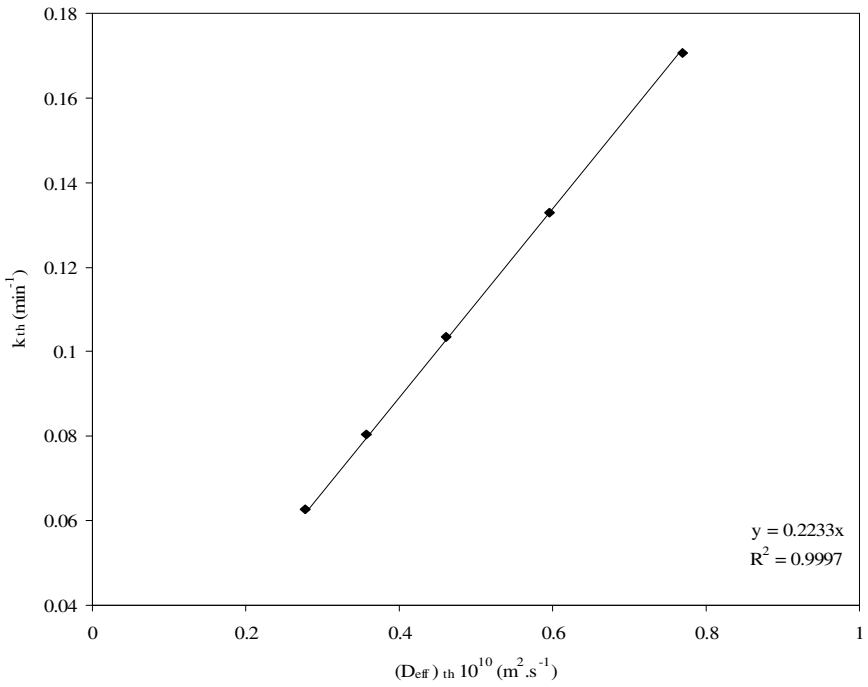

Figure 4
Relationship between drying rate constant, k_{th} and effective moisture diffusivity (D_{eff})$_{th}$ for microwave drying of Adhatoda vasica.

Comparison of yields obtained by MAE and MSE

In the extraction of bioactive components from plants , besides free diffusion, exterior functions like microwave irradiation, ultra sound, etc., also contribute to the reduction of mass transfer resistances and produce an enormous impact on the extraction process[37]. To investigate the effect of sample mass, volume of solvent and microwave output power, three different compositions of sample mass to solvent ratio were used (1:10, 1:15 and 1:20) during extraction. Similarly, for microwave drying of samples (pre-processing) three MW output power (100, 180 and 300 W) and five sample mass (10, 20, 30, 40 and 50 g) were considered.

Effect of sample mass during preprocessing

When the sample mass was increased from 10 to 50 g, for a constant solid : solvent ratio (1:10) with microwave output power (180 W), the results of extraction yield dropped significantly from 33.66 to 12.66 % for MSE and from 29.98 to 05.52 %, 28.02 to 04.35 % and 26.65 to 03.53 % for 100, 180 and 300 W respectively under MAE. In all cases of microwave drying, the extraction yield was higher for the 10 g followed by 20, 30, 40 and 50 g, irrespective of the MW output power that is used for drying (Table 3).

Effect of Solid to Solvent ratio under MSE and MAE

During extraction, while the sample mass to solvent ratio was raised from 1:10 to 1:20, the extract yield obtained also increased from 36.11 to 40.28 % for MSE and from 32.72 to 37.83 %, 30.66 to 34.64 % and 29.25 to 32.71 %, for MAE at 100, 180 and 300 W respectively. The increase in the extraction yield may be due to the fact that the solvents used (Hexane in MSE and mixed solvent in MAE) had effectively increased the contact surface area between the plant matrix and the solvent.

Effect of Microwave output power under MSE and MAE

During MAE, the extraction yield was reduced on increasing the MW output power from 100 to 300W irrespective of the preprocessing conditions and sample to solvent ratio. However, higher MW output power has also been associated with physical damages to the products during MAE, like scorching, over-heating, charring and uneven temperature distribution. Such physical damages are the result of continuous local temperature rise even though the loss of the material being irradiated decreases with reduction in the moisture content. This also indicated a possible non-uniform distribution of MW inside the cavity. Hence it is difficult to recover more extracts from the pre-processed *A. vasica* when MW output power used was more than 100 W. The results obtained in the present study was not in agreement with the results obtained during the recovery of phenolic compounds from rosemary leaves, where lower as well as higher MW output power has produced higher recoveries[38]. The MSE process had an average extraction time of 10 hours and produced a higher yield of 6.082 % when compared to MAE, which took 1178, 460 and 210 sec for 100, 180 and 300 W extraction experiments respectively. The yield of extract obtained from samples subjected to MSE was significantly higher than MAE, irrespective of MW output power, sample mass and solvent used (Table 3). This result could be explained by the fact that, Soxhlet extraction was carried out for 10 hours, which led to more contact time between the solvent and the plant matrix. This enabled the complete diffusion of the solvent and dissolution of the extracted substances present in the plant matrix into the solvent. Despite good results obtained with MSE, the proposed MAE method has significant advantages in extraction efficiencies and short processing time. Moreover, the microwave energy applied acted as a major factor (rise in temperature and the internal pressure) to influence the release of target compounds (vasicine and vasicinone). It must also be noted that microwave energy has interacted selectively with the dipolar molecules present in both the mixed extraction solvent and *A. vasica* matrix. The results are in agreement with those of Pan et al.,[39] who studied MAE of polyphenols and caffeine extraction from green tea leaves in comparison with Soxhlet extraction.

Table 3

Percentage yield of extract obtained by MSE and MAE from Microwave dried Adathoda vasica.

Sample No.	Microwave drying		Extraction				
	Microwave output power, W	Mass of Sample, g	Sample: Solvent	Yield of Extract, %			
				Microwave Assisted Soxhlet	Microwave Assisted Extraction		
					100 W	180 W	300 W
1	100	10	1:10	36.11	32.72	30.66	29.25
2			1:15	38.47	33.15	30.45	28.85
3			1:20	**40.28**	**37.83**	**34.64**	**32.71**
4		20	1:10	34.78	29.89	27.95	26.75
5			1:15	35.76	31.07	28.60	27.09
6			1:20	38.24	33.60	30.51	28.75
7		30	1:10	30.38	23.29	21.42	20.29
8			1:15	31.02	24.45	22.05	20.58
9			1:20	33.79	27.83	24.87	23.17
10		40	1:10	21.47	12.08	10.29	09.22
11			1:15	22.89	13.76	11.39	09.99
12			1:20	24.09	15.49	12.67	11.03
13		50	1:10	16.34	07.91	06.31	05.29
14			1:15	17.10	09.34	06.99	05.65
15			1:20	19.74	09.98	07.24	05.67
16	180	10	1:10	33.66	29.98	28.02	26.65
17			1:15	34.91	30.84	28.28	26.83
18			1:20	36.62	33.21	30.13	28.29
19		20	1:10	31.75	25.81	23.94	22.69
20			1:15	32.12	27.44	25.22	23.84
21			1:20	34.96	30.89	27.92	26.23
22		30	1:10	27.62	20.33	18.79	17.73
23			1:15	28.90	22.64	20.51	19.13
24			1:20	30.66	23.28	20.54	18.92
25		40	1:10	19.23	09.93	08.54	07.57
26			1:15	20.14	10.77	08.73	07.48
27			1:20	21.89	12.63	10.03	08.61
28		50	1:10	12.66	05.52	04.35	03.53
29			1:15	13.77	07.30	05.38	04.34
30			1:20	15.02	08.56	06.34	05.22
31	300 W	10	1:10	32.79	28.94	27.25	26.01
32			1:15	33.88	29.43	27.11	25.81
33			1:20	35.05	31.45	28.59	26.88
34		20	1:10	30.06	25.56	23.92	22.81
35			1:15	31.87	26.84	24.77	23.54
36			1:20	33.98	28.22	25.41	24.02
37		30	1:10	26.78	19.98	18.56	17.52

38		1:15	27.43	20.74	18.78	17.62
39		1:20	29.05	22.02	19.48	18.18
40	40	1:10	18.45	08.32	07.02	06.09
41		1:15	19.18	10.62	08.88	07.84
42		1:20	20.79	11.78	09.41	08.19
43	50	1:10	11.02	03.95	02.68	01.86
44		1:15	12.87	04.08	02.79	01.94
45		1:20	13.31	05.89	03.78	02.10

Characterization

The effects of different drying methods and conditions on the structure of dried *A. vasica* leaves were observed under SEM. As shown in Fig. 5, the SEM micrograph of the cross-sections of *A. vasica* leaves, MW irradiation caused large changes in the microstructure in comparison to natural open-air sun dried samples. The absence of open structure and pores in Fig. 5a indicated severe tissue shrinkage and collapse during dehydration, resulting in a low quality structure (open air sun drying). Conversely, the more porous structure in MW drying was possible from massive and fast vaporization during MW irradiation (Fig. 5b). MW created a large vapor pressure in the centre of the leaf, allowing rapid transfer of moisture to the surrounding vacuum and preventing a structural collapse. It could be presumed that the energy of MW is absorbed by water located in the whole volume of the material being dried[40]. The microstructure clearly shows that the heat was mainly generated in the bulk of the sample and transported from the bulk to the environment. This type of temperature gradient can only be generated by MW heating. Above all, the spore stalk matrix emerging from the stoma and guard cells on the epidermis of leaves was clearly visible (Fig. 5b).

Scanning electron micrographs of natural Adathoda vasica leaves: (a) Untreated leaves (1200X)

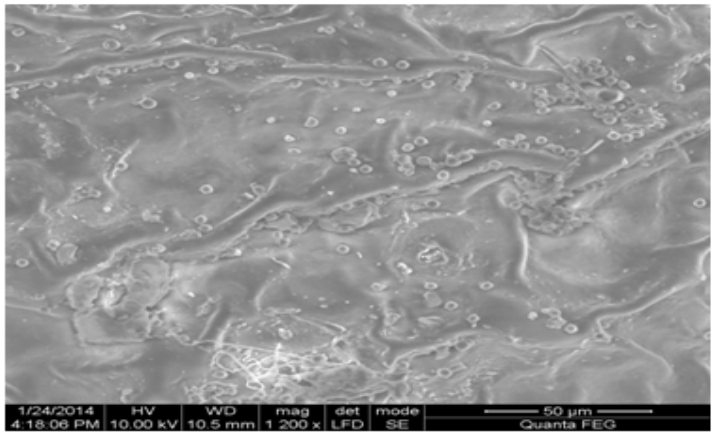

Figure 5(a)
Scanning electron micrographs of natural and microwave treated (100W) Adathoda vasica leaves: Untreated leaves (1200X).

Scanning electron micrographs microwave treated (100W) Adathoda vasica leaves (b) Treated leaves showing spore stalk matrix from stoma (1200X).

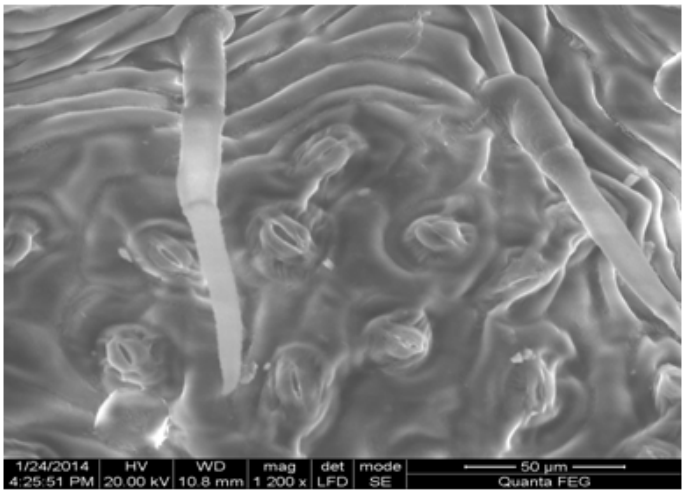

Figure 5(b)

Scanning electron micrographs of microwave treated (100W) Adathoda vasica leaves: Treated leaves showing spore stalk matrix from stoma (1200X).

IR spectrogram of extract obtained from Adathoda vasica under different microwave output power (a) 100 W; (b) 180 W; (c) 300 W.

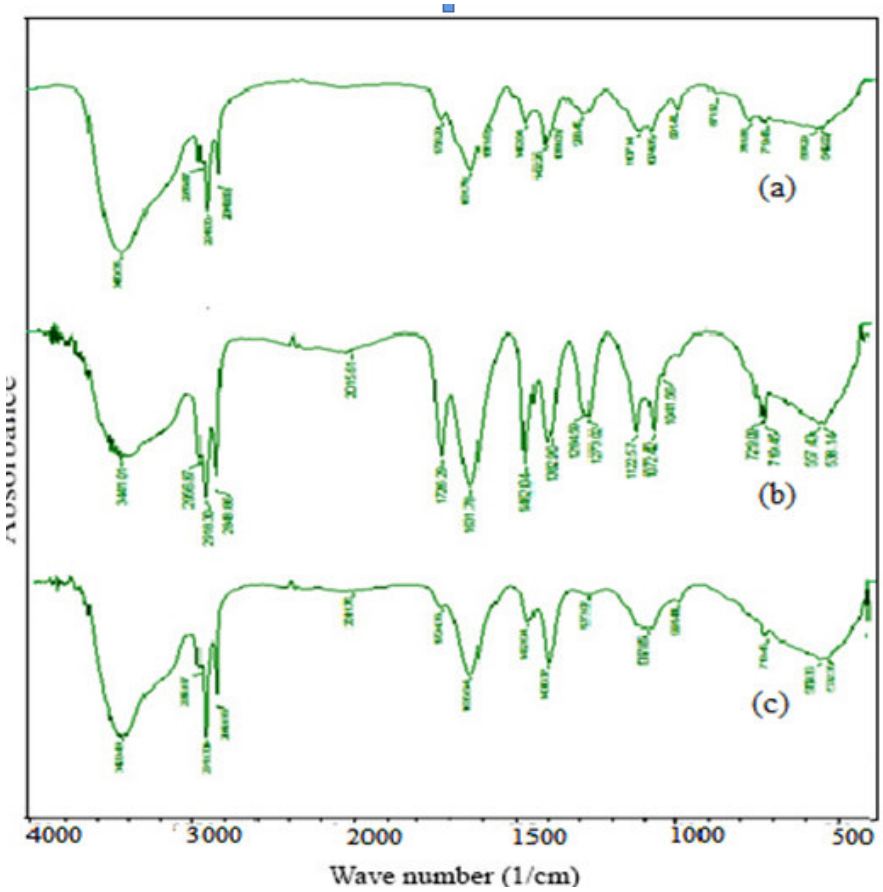

Figure 6

IR spectrogram of extract obtained from Adathoda vasica under different microwave output power (a) 100 W; (b) 180 W; (c) 300 W.

CONCLUSION

The effect of sample pre-treatment prior to MAE and MSE on the extractability of bioactive components from *A. vasica* leaves was investigated. The most important factors that influenced MW drying (pre-treatment) were found to be the sample mass and the MW output power. The newly developed model equation adequately explained the thin layer MW drying behavior confirming the suitability of the developed model. The yield of extract obtained from samples subjected to MSE was significantly higher than MAE, irrespective of MW output power, sample mass and solvent used, for both the extraction methods. Although MSE gave a better yield than MAE, the extraction time in the latter was significantly lesser by 28.70 fold (100 W), 67.31 fold (180 W) and 139.20 fold (300 W), proving that MW output power had a crucial effect on the rate of extraction. Hence, it may be concluded that MW drying, followed by MAE could greatly increase the extraction rate, reduce the drying time and can successfully be used to extract the bioactive compounds from *A. vasica* leaves. This study has given wide scope to harness MW for efficient and cost effective extraction of bioactive components. A detailed scale-up process may be undertaken to develop a viable method for high throughput extraction.

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CONFLICT OF INTEREST

Conflict of interest declared none.

NOMENCLATURE

a, a_1,b, b_1,c, g, k, k_1, k_2, n, n_1	:	Empirical coefficients
D_{eff}	:	Effective moisture diffusivity [$m^2.sec^{-1}$]
D_o	:	Pre- exponential factor [$m^2.sec^{-1}$]
E_a	:	Activation energy [$W.g^{-1}$]
k_o	:	Pre- exponential factor [min^{-1}]
L	:	Half the thickness of the sample [m]
m	:	Sample mass [g]
M	:	Number of mathematical models used for empirical modeling.
MC_i	:	Moisture content at specific time [g water/ g dry solids]
MC_o	:	Moisture content at initial time [g water / g dry solids]
MR	:	Moisture ratio
N	:	Number of model constants for each equation as in Table 1
P	:	Microwave output power [W]
R^2	:	Coefficient of determination
E_{RMS}	:	Root mean square error
RSS	:	Residual sum of squares

t	:	Drying time [min]
W_c	:	Weights of the crude extract [g]
W_o	:	Weight of dry powdered sample [g]

Greek symbols

α	:	Constant [$min^{-1}/m^2\ sec^{-1}$]
η_m	:	Modeling efficiency
χ^2	:	Chi-square

Subscripts

o	:	Initial
i	:	Specific time
th	:	Theoretical

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