Multi-step reflux extraction of bio-pharmaceutical phenolic bioactives from balsam apple (Momordica balsamina L.)

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ABSTRACT
In this study, the impacts of parameter combinations on the response (total phenolic yield) were evaluated using the single experiments and two-level factorial design. The investigated parameters with their value range include; the microwave extraction time (1–5 min), power level (400–600 W), temperature (60–80°C), the solvent-feed ratio, SFR (8–12 mL/g) and solvent concentration (50–80%). The result of this investigation succinctly presented the solvent concentration as the highest contributing parameters, far above the Bonferroni limit (8.37517) and t-value limit (3.18245). However, the temperature, SFR and microwave duration provided insignificant contribution to the response settings. The result of the spectrometry chromatographic analysis identified 22 phenolics compounds from the extracts which invariably revealed their potential biological functions. The study therefore adequately elucidated the contributing factor in the microwave recovery of total phenolic constituents from the M. balsamina leaves with potential use as scale-up parameters in drug applications.

1. Introduction
In recent times there has been a renewed interest in the study of major phenolics content in medicinal plants. Evidence from traditional medicine practitioners confirmed that frequent consumption of these plants could mitigate many life-threatening diseases which is a safe alternative to the use of synthetic drugs [1,2]. It is pertinent to note that tropical regions are vastly enriched with varieties of medicinal plants with potential application in pharmaceutical and food industries [3]. Their inherent medicinal properties are largely due to the abundance of different groups of bioactive content with remarkable physiological actions on human body. Phenolics constituent is an example of medicinal components found in many medicinal and functional plants with diverse health benefits [4]. This group of compounds is largely responsible for the antioxidant activities of many plant products as reported by Niroula et al. [4]. To, therefore, determine the amount of phenolics compounds inside the plant extracts, the microwave-refluxing is a viable green extraction method for achieving an efficient process [4].

Due to environmental effects and inherent health issues, the use of green extraction process has received a lot of attention lately [5]. The application of an economically competitive extraction process has found its use in food, pharmaceutical, chemical, cosmetic and agro-allied industries [6]. More than ever before, many researchers had emphasized the use of solvent-free green extraction technology for an efficient recovery of phenolics metabolites in both functional and medicinal plants [7]. The application of green extraction procedures such as microwave technique, supercritical fluid extraction and ultrasound extraction is attributable to their excellent performance in terms of cleaner process, cost effectiveness, safe and ease of operation as buttressed by Chemat et al. [5]. Microwave reflux extraction method is a viable green-extraction technique with a multi-step intermittent heating and pulse electromagnetic radiation. The observable cooling counter-current effect in microwave refluxation has been reported to generate less contaminant, higher quality extracts and lesser solvent consumption when compared to other green method of extraction as enumerated by Kazmi et al. [6].

Moreover, the high sensitivity and stability of the phenolic content to heat degradation and deterioration are important factors to consider in selecting the microwave extraction technique [7]. Olalere et al. [8] investigated the degradation of the plant matrix under microwave extraction at molecular and structural level. The result obtained confirmed a positive textural and dispersive transformation in the microchemical structure as a result of the intermittent microwave electromagnetic probing. Benkaci-Ali et al. [9] reported...
that the cooling effects from the microwave refluxing mitigate the superheating which in-turn minimize the degradation of heat-sensitive phenolics metabolites in the plant matrix.

Furthermore, the complexity of the heat and mass transfer phenomenon has been regarded as the most striking set-back in the process of scaling-up the microwave extraction of plant-based products [10]. The challenge of scaling-up in microwave extraction is largely related to the variability, specificity and non-deterministic nature of the extraction conditions for different models of microwave system [10]. The industrialization of microwave system therefore requires the determination of suitable extraction condition. The need then arises to study the effect of parameters which will invariably help in predicting the behaviour of the extraction system.

*Momordica balsamina* Linn is a vegetable from the family of Cucurbitaceae and is grown mainly in tropical India, China, East Africa, West Africa and South America [11]. Moreover, besides its nutritional benefits which have made it widely consumed in many parts of Africa, it has also been used traditionally for the treatment of malaria and fever. It is a climber or trailer plant with stems usually grown up to a multi-branching stem network having an average length of about 4–5 mm. There are up to 15 species which is usually grown because of the high proteinous seeds and flavoured leaves. The preliminary research on the phytochemical constituents has shown the presence of 13 important phenolics phyto compounds that are largely responsible for its antioxidant, antimalarial activities and other medicinal benefits [11]. *M. balsamina* has also been reported to have other benefits such as anticancer, hypocholesterolemic as reported by Ronny and Navam [12]. Balsam apple is a rich source of phenolics phyto compounds, which has been revealed by many researchers to exhibit health-promoting properties such as the reduction of blood sugar, cancer and some cardiovascular diseases [13,14].

Many researchers had reported the phenolics content in balsam apple with potential health benefits such as antioxidant activity with an inherent capacity to eliminate free radical cells in the body. However, information on the phenolics content of balsam apple (*M. balsamina*) is limited and has not been fully harnessed at the experimental design level. Many studies investigated the biological activities of *M. balsamina* without the application of design of experiment (DOE) to study the changes in parameters effects on the total recovery of the phenolics constituents using microwave-reflux extraction techniques. A more detailed investigation is therefore required to determine the effects of parameter changes on the total phenolics contents and provides information for their nutraceutical values. Five microwave extraction parameters were studied to determine their influence on the TPC content in *M. balsamina* using the one-factor-at-a-time (OFAT) and two-level factorial experimental design.

### 2. Materials and methods

#### 2.1. Extract preparation and design of experiment

*M. balsamina* leaves were collected and dried in an open tray. These were subsequently grounded to uniform mean particle size of 0.1 mm. Moreover, reagents such as analytical ethanol (99.5% purity), distilled water, sodium carbonate and Folin–Ciocalteu were all supplied by Sigma Aldrich Chemical Co without further purification. Microwave reflux extractor (ATC-FO-300 model, North. America) was employed to prepare the *M. balsamina* leaves extracts. Briefly, 5 g of *M. balsamina* sample was extracted using aqueous ethanol in a microwave round bottom reactor. The Milestone extraction has three heating levels viz: pre-heating at 80°C for 2 min, irradiation at designing matrix settings, and cooling to 30°C for 2 min. The parameters setting was used based on the one-factor-at-time (OFAT) and two-level factorial experimental designs. The product obtained from the reactor was thereafter filtered and concentrated with a rotary evaporator (Buchi-200, Germany) and stored at 4°C prior to further analysis.

One-factor-at-a-time is an important, iterative design used in this study to investigate the effect of parameter changes on the TPC. This was carried out varying one factor, other factors set as constant parameters. The results obtained from the trend of values in the OFAT experiments were thereafter used to search for a range of values in the subsequent two-level factorial design using Design Expert 7.0 software®. In both designs five (5) factors were investigated via microwave extraction time (A), microwave power (B), temperature (C), the solvent-feed ratio SFR (D) and ethanol concentration (E). The best extraction condition was obtained based on the largest donating rule using the one-factor-at-a-time array.

#### 2.2. Total phenolics content

Folin–Ciocalteu calorimetric method was employed to estimate the TPC using the method reported by Olalere et al., [8]. Briefly, 1.58 mL of distilled water was mixed with 20 µL of liquid *M. balsamina* extract. Folin–Ciocalteu’s phenol reagent (100 µL), was thereafter added and the resulting mixture was incubated for an initial period of 5 min. After that, 300 µL of sodium carbonate solution was added to the mixture and incubated at 25°C for 2 h. UV-VIS Spectrophotometer (Hitachi U-1800, Japan) was employed to measure the absorbance at 765 nm.

Gallic acid was used as a standard for the calibration curve to determine the total phenolic concentration of the oleoresin extracts expressed in mg/L gallic acid.
The total phenolic contents were calculated in triplicates using Equation (1).

\[
TPC = \frac{c(mg\text{GAE}/L \times V(L_{Solvent}))}{m(\text{gdw dryweight})}
\]

where \(c\) is the sample concentration in from the calibration curve (mg/L), \(V\) is the volume (mL) of the solvent used in the extraction and \(m\) represents the weight (g) of the dried sample used.

2.3. LCMS QToF phenolics profiling

The liquid chromatography (LC) quadrupled time of flight mass spectrometer (QToF-MS) is an excellent technique for accurate mass measurement, fragmentation and selectivity of targeted and non-targeted bioactive compounds in polar and non-polar natural products. This technique works with higher resolution power above ten-thousand FWHM (Full peak width at one-half maximum) which explains their ability to give an accurate mass measurement of parent and fragmented ions which is a major advantage over gas Chromatography-Mass Spectrometry (GCMS). In this study, the use of a quadrupled-ToF-mass spectrometer succinctly provides a higher degree of selectivity and detail structural information about the analysed samples. LC-MS has the ability to incorporate several mass analyzers with an unequalled capacity for component identification and mass quantification with suitable ionization and acquisition parameters. The parameters used in this analysis are in accordance with Olalere et al. [15].

3. Results and discussion

3.1. Results from one-factor-at-a-time experimental design

3.1.1. Effect of extraction duration

Figure 1 showed a steady rise in the total phenolics between (from 1 to 2 min) up to 5 min of threshold microwave duration. At this peak, the TPC recorded as 102.01 ± 0.02 mg GAE g\(^{-1}\), which later decreases to the lowest value (44.14 ± 0.003 mg GAE g\(^{-1}\)) after 15 min of extraction. This result indicated that an increase in extraction time beyond 5 min will lead to the degradation of the phenolics and this is consistent with the investigation made when phenolics content was evaluated in Vernonia amygdalina and Sesamum indicum as reported by Alara et al. [16] and Olalere et al. [14], respectively.

3.1.2. Effect of microwave power

In Figure 2, the power level variation shows an initial increase in the TPC from 55.33 ± 0.04 to 108.29 ± 0.003 mg GAE g\(^{-1}\), varied from 200 to 600 W. The TPC, however, experienced a downward trend and dropped sharply to an all low value of 30.55 ± 0.02 mg GAEG\(^{-1}\) at a constant temperature of 40°C, microwave duration of 2 min, molar ratio of 10 mL g\(^{-1}\) and ethanol concentration of 20. This suggested a corresponding denaturing of the phenolics constituents in M. balsamina due to the high electromagnetic heating [11].

3.1.3. Effect of temperature

Figure 3 shows the results from temperature variation at constant extraction time of 2 min, microwave power of 600 W, the SFR of 10 mL g\(^{-1}\) and ethanol concentration of 20%. A higher TPC value was attained with a threshold temperature of 80°C at 102.59 ± 0.12 mg GAE g\(^{-1}\). The total phenolics content however reduced to 44.24 ± 0.08 mg GAE g\(^{-1}\) when the reactor temperature was increased to 100°C. At higher reactor temperature the phenolics inside the M. balsamina L. sample could not withstand the heat generation which leads to an eventual degradation to 44.24 ± 0.08 mg GAE g\(^{-1}\). This is consistent with an investigation conducted by Rodsraman and Sothornvit [17] who reported that at a temperature above the boiling temperature of the extracting solvent there is a high tendency for degradation of the phenolics in a plant sample.

3.1.4. Effect of SFR

Figure 4 shows the SFR variation from 6 to 14 mL g\(^{-1}\) at a constant temperature of 80°C, extraction time of 2 min, microwave power of 600 W and ethanol concentration of 20%. The total phenolics content increased from 55.13 ± 0.003 to 110.29 ± 0.011 mg GAE g\(^{-1}\) and later dropped to 60.25 mg GAE g\(^{-1}\). This suggests an increase in solvent evaporation which leads to a
higher heat generation and this is responsible for the degradation in the phenolics as supported by Deng et al. [18].

3.1.5. Effect of ethanol concentration

Figure 5 shows the variation of ethanol concentration 20–80% at constant extraction time of 2 min, microwave power of 600 W, the molar ratio of 10 mL g\(^{-1}\) and a reactor temperature of 80°C. From the result obtained the total phenolics content increased steadily from 46.04 mg GAE/g d.w to 102.91 mg GAE/g d.w as the ethanol concentration respectively increased from 20% to 60%. This result indicated that a value beyond the 60% of extracting solvent, the total phenolics reduces and this is consistent with the result obtained by who reported Alara et al., [16] a threshold 60% ethanol concentration beyond which the total phenolics content decreases in microwave extraction of Vernonia amygdalina leaf.

### Table 1. Extraction parameters for the two-level factorial design.

| Extraction variables | Units | Notation | Low (−1) | High (+1) |
|----------------------|-------|----------|----------|-----------|
| Extraction time      | Min   | A        | 1        | 5         |
| Microwave power      | W     | B        | 400      | 600       |
| Reactor temperature  | °C    | C        | 60       | 80        |
| SFR                  | mL/g  | D        | 8        | 12        |
| Ethanol concentration | %    | E        | 50       | 80        |

### Table 2. Experimental matrix and results obtained from two-level factorial design

| Run | A (min) | B (W) | C (°C) | D (mL/g) | E (%) | TPC (mg GAE/g d.w) |
|-----|---------|-------|--------|----------|-------|-------------------|
| 1   | 1       | 400   | 60     | 8        | 80    | 103.68            |
| 2   | 5       | 400   | 60     | 8        | 50    | 99.38             |
| 3   | 1       | 800   | 60     | 8        | 50    | 101.00            |
| 4   | 5       | 800   | 60     | 8        | 80    | 103.23            |
| 5   | 1       | 400   | 90     | 8        | 50    | 107.13            |
| 6   | 5       | 400   | 90     | 8        | 80    | 101.29            |
| 7   | 1       | 800   | 90     | 8        | 50    | 97.90             |
| 8   | 5       | 800   | 90     | 8        | 80    | 97.99             |
| 9   | 1       | 400   | 60     | 12       | 50    | 104.21            |
| 10  | 5       | 400   | 60     | 12       | 80    | 107.55            |
| 11  | 1       | 800   | 60     | 12       | 80    | 90.22             |
| 12  | 5       | 800   | 60     | 12       | 50    | 103.54            |
| 13  | 1       | 400   | 90     | 12       | 80    | 99.35             |
| 14  | 5       | 400   | 90     | 12       | 50    | 100.57            |
| 15  | 1       | 800   | 90     | 12       | 50    | 99.35             |
| 16  | 5       | 800   | 90     | 12       | 80    | 98.99             |

The summary of the selected parameter levels is presented in Table 1.

### 3.2. Statistical analysis

A total of 16 trials were generated in this design to evaluate the degree of contribution of each factor to the response settings. The results investigated showed a significant model as presented in Table 2. The bar length of the Pareto chart is directly proportional to the absolute value of the estimated main effects of each extraction parameters with a 99% confidence level (i.e. \(p < 0.05\)). The result obtained demonstrated that the ethanol concentration has the most significant effects on the total phenolics content of M. balsamina leaf, while the interaction between the microwave power and the reactor temperature was almost half of their effects.

As presented in Figure 6, the most contributing factors have the Pareto bar length above the Bonferroni limit of 8.37517 and \(t\)-value limit of 3.18245. The higher level of significance of the ethanol concentration suggested the higher affinity of the extracting solvent for the total phenols yield in the sample matrix [19].

It is pertinent to note that the use of water–ethanol mixture improved the energy absorption due to the increase in the absorbed energy which in turn led to an increase in the TPC in the M. balsamina L. [20]. On the
Figure 6. Pareto chart showing the overall parametric screening effect.

Table 3. Analysis of variance ANOVA.

| Model term          | Sum of squares | Mean square | F-value | p-value | Prob > F |
|---------------------|----------------|-------------|---------|---------|----------|
| Model               | 784.52         | 60.35       | 53.91   | 0.0184  | a        |
| A-Extraction time   | 6.03           | 6.03        | 5.38    | 0.1461  | b        |
| B-Microwave power   | 41.09          | 41.09       | 36.70   | 0.0262  | b        |
| C-Temperature       | 20.88          | 20.88       | 18.66   | 0.0496  | b        |
| D-SFR               | 28             | 28          | 25      | 0.6689  |          |
| E-Ethanol concntr.  | 393.02         | 393.02      | 352.86  | 0.0028  | a        |
| AB                  | 27.98          | 27.98       | 25.00   | 0.0378  | b        |
| AD                  | 22.42          | 22.42       | 20.03   | 0.0465  | b        |
| BC                  | 96.92          | 96.92       | 86.58   | 0.0114  |          |
| BD                  | 12.46          | 12.46       | 11.13   | 0.0793  |          |
| BE                  | 8.18           | 8.18        | 7.31    | 0.1139  |          |
| CD                  | 56.25          | 56.25       | 50.25   | 0.0193  |          |
| CE                  | 86.68          | 86.68       | 77.43   | 0.0127  |          |
| DE                  | 10.34          | 10.34       | 9.23    | 0.0934  |          |
| Residual            | 2.24           | 1.12        |         |         |          |
| Cor Total           | 786.76         |             |         |         |          |

*p*Significant, *b*Not significant.

overall, the ethanol concentration and the microwave power were the only significant factors (*p* < 0.05), while extraction time, temperature, SFR were the insignificant factors. Moreover, the interaction such as BC, CE, CD, AB and AD significantly affect the TPC in *M. balsamina* leaf extraction. However, interactions such as BD, DE and BE were insignificant interactions to the extraction of phenolics from *M. balsamina* leaf as presented in Table 3.

Moreover, the best extraction condition was selected in accordance with the largest donating principle, which states that as far as the investigated parameters are concerned, the response factors with the largest value are selected as the optimum response [8]. In Table 4, the summary of the best microwave extraction conditions was presented. The extraction condition was validated by conducting a triplicate test. From the parallel trials for the microwave reflux extraction of *M. balsamina* leaves the total phenolics content recovery of 112.06, 111.56 and 112.07 mg GAE/g d.w. were obtained. According to Alara et al. [16], the estimated X-goodness-of-fit indicated an insignificant difference between the observed and predicted TPC (112.07 mg GAE/g d.w.). Thus, the $X^2$-value was lower than the cut-off value (7.81) at a *p* < 0.005 for a three-degree of freedom.

### 3.3. Phenolics profiles of microwaved Momordica balsamina extract obtained by MAE

The phenolics profile of the *M. balsamina* extracts was observed under the best extraction condition of 5 min extraction time, 600 W power level, 80°C oven temperature, 10 mL/g SFR and 60% solvent concentration. A total of 22 phenolics constituents were were tentatively identified from the *M. balsamina* extracts (Table 5) and the representative compounds illustrated in Figure 7(a,b). The results obtained revealed four important biomarkers such as geraniin (m/z = 997.08; $R_t$ = 18.53 min), tellimagrandin II (m/z = 983.10; $R_t$ = 17.86 min), 1-O-galloylpedunculagin (m/z = 981.09; $R_t$ = 18.53 min) and terchebin (m/z = 953.09; $R_t$ = 17.86 min). The results from the mass-to-charge ratio revealed geraniin ($C_{41}H_{28}O_{27}$) as the most abundant phenolics compounds with more antioxidant and anti-inflamatory pharmacological health benefits as butressed by Yeh et al. [21]. Yeh et al. [21] reported the inhibitory capacity of geraniin on oral cancer cell migration through the suppression of matrix metalloproteinase-2 activation using the FAK/Src and ERK as biological pathways. Moreover, the second most abundant phenolics constituent was tellimagrandin II ($C_{41}H_{30}O_{26}$) with inherent anticancer capacity. Yi et al. [22] investigated the inhibitory effect of tel-limagrandin on chemically induced differentiation of human leukaemia K562 cells. Tercbebin ($C_{41}H_{30}O_{27}$) and 1-O-galloylpedunculagin ($C_{41}H_{28}O_{26}$) are phenolics constituents from the *M. balsamina* extracts with antioxidant activities as reported by Richman et al. [23] and Tanimura et al. [24]. Hence, the cumulative bioactivities of these phenolics constituents are largely responsible for the numerous therapeutic functions of *M. balsamina* as reported by many researchers and presented in Table 5. The result described from this investigation can therefore be used for further studies into other nutraceutical applications of *M. balsamina* extracts.

Table 4. Best extraction condition based on the largest donating rule.

| Extraction variables | Units | Notation | Best condition |
|----------------------|-------|----------|----------------|
| Extraction time      | min   | A        | 5              |
| *Microwave power*    | W     | B        | 600            |
| Reactor temperature  | °C    | C        | 80             |
| SFR                  | mL/g  | D        | 10             |
| *Ethanol concntr.*   | %     | E        | 60             |
| Total phenolics content | mg GAE/g d.w. | TPC | 112.07         |

*a*Most contributing factor.
Figure 7. LCMS-Q-ToF chromatogram of *M. balsamina* extracts (a) BPI plot (b) confirmed phenolic compounds.

Table 5. Tentative phenolics profile and pharmacological properties of *Momordica balsamina* extracts.

| Identified compounds | Chemical Formula | Observed m/z | Observed Rt (min) | Adducts | Pharmacological activities | References |
|----------------------|------------------|--------------|-------------------|---------|---------------------------|------------|
| Tribulusamide B      | C\textsubscript{36}H\textsubscript{34}N\textsubscript{2}O\textsubscript{9} | 683.22       | 0.39              | +HCOO   | anti-inflammatory          | Lee et al. [25] |
| Yakuchinone A         | C\textsubscript{20}H\textsubscript{24}O\textsubscript{3} | 311.16       | 0.40              | -H      | antioxidant, anti-cancer   | Chun et al. [26]; Sethi et al. [27] |
| Methyl-5-O-caffeoylquinic | C\textsubscript{17}H\textsubscript{20}O\textsubscript{9} | 367.10       | 0.41              | -H      | antioxidant               | Jayasinghe et al. [28] |
| Thannilignan          | C\textsubscript{19}H\textsubscript{22}O\textsubscript{5} | 329.13       | 9.72              | -H      | antioxidant, anti-cancer, anti-hepatitis | Majumder et al. [29] |
| 1,7-Bis(4-hydroxyphenyl)-hepta-4E,6E-dien-3-one | C\textsubscript{19}H\textsubscript{18}O\textsubscript{3} | 339.12       | 10.08             | +HCOO   | anti-neuroinflammatory     | Woo et al. [30] |
| Obovatol              | C\textsubscript{19}H\textsubscript{20}O\textsubscript{3} | 327.12       | 10.08             | +HCOO   | anti-neuroinflammatory     | Ock et al. [31] |
| Pseudoaspidin         | C\textsubscript{20}H\textsubscript{22}O\textsubscript{6} | 505.20       | 10.43             | +HCOO   | anti-bacterial, antioxidant | Deng et al. [32] |
| Agrimol E             | C\textsubscript{19}H\textsubscript{18}O\textsubscript{12} | 625.23       | 11.12             | -H      | antioxidant, anti- inflammatory | Yin et al. [33] |
| Decaffeoylacteoside   | C\textsubscript{20}H\textsubscript{20}O\textsubscript{12} | 461.16       | 16.47             | -H      | antioxidant, anti- inflammatory | Kim et al. [34] |
| Kukoamine A           | C\textsubscript{28}H\textsubscript{42}N\textsubscript{4}O\textsubscript{8} | 529.30       | 16.48             | -H      | antioxidant               | Chahel et al [35] |
| Geraniin              | C\textsubscript{20}H\textsubscript{24}O\textsubscript{2} | 997.07       | 17.02             | +HCOO   | antioxidant, anti- inflammatory | Yeh et al. [21] |
| Kuzubutenolide A      | C\textsubscript{32}H\textsubscript{24}O\textsubscript{10} | 459.12       | 17.04             | -H      | anti-inflammatory          | Xue et al. [36] |
| 1,2,3,6-Tetra-O-galloyl-β-D-glucopyranoside | C\textsubscript{27}H\textsubscript{28}O\textsubscript{12} | 787.09       | 17.64             | -H      | antioxidant, cytotoxic and antimicrobial activities | Zhou et al. [37] |
| Mallotinic acid       | C\textsubscript{34}H\textsubscript{26}O\textsubscript{22} | 785.08       | 17.81             | -H      | antioxidant, anti- inflammatory | Yang et al. [38] |
| Tellimagrandin II     | C\textsubscript{34}H\textsubscript{32}O\textsubscript{26} | 983.10       | 17.86             | +HCOO   | anti-cancer               | Yi et al. [22] |
| Terchebin            | C\textsubscript{34}H\textsubscript{32}O\textsubscript{17} | 953.09       | 18.51             | -H      | antioxidant               | Richman et al. [23] |
| Laevigatin A          | C\textsubscript{34}H\textsubscript{24}O\textsubscript{13} | 847.08       | 18.51             | +HCOO   | antioxidant               | Fecka et al. [39] |
| 2,7-Dihydroxy-4-methoxyphenanthrene-2-O-glucoside | C\textsubscript{42}H\textsubscript{28}O\textsubscript{14} | 447.13       | 18.52             | +HCOO   | anti-cancer               | Ukaegbue et al. [40] |
| Yakuchinone A         | C\textsubscript{20}H\textsubscript{24}O\textsubscript{2} | 357.17       | 18.52             | +HCOO   | anti-angiogenic            | He et al. [41] |
| Flavanthrinin         | C\textsubscript{13}H\textsubscript{12}O\textsubscript{3} | 285.07       | 18.53             | +HCOO   | antimarial and anti-herpetic | Sukphan et al. [42] |
| 1-O-Galloylpedunculagin | C\textsubscript{20}H\textsubscript{24}O\textsubscript{12} | 981.08       | 18.53             | +HCOO   | antioxidant, anti-inflammatory | Tanimura et al. [24] |
| 2,4,6-Trihydroxyacetophenone-2,4-di-O-β-D-glucopyranoside | C\textsubscript{20}H\textsubscript{24}O\textsubscript{14} | 537.14       | 18.53             | +HCOO   | hepatoprotective          | Fecka et al. [39] |
4. Conclusion

This study entails the contributory effects of five microwave parameters on the total phenolics recovery from *M. balsamina* leaves. To achieve this, one-factor-at-a-time factor and two-level factorial designs were employed to study the effects of parameter changes on the response settings. The results obtained from the investigated single factor experiments presented factor ranges for the extraction time (1–5 min), microwave power (400–600 W), temperature (60–80°C), the SFR (8–12 mL/g) and ethanol concentration (50–80%). The power level and solvent concentration have significant contribution with \( p < 0.05 \), while temperature, molar ratio and microwave duration are insignificant factors in the extraction of *M. balsamina* L. The best extraction condition was achieved at 5 min of microwave duration, 600 W of microwave power level, 80°C of reactor temperature, 10 mL/g of molar ratio and 60% of solvent concentration. A total of 22 polyphenolics constituents were identified from the *M. balsamina* extracts with their biological functions. The overall bioactivities and phenolics characterization constituents of the extracts are largely responsible for the numerous therapeutic functions of *M. balsamina* L. as reported by many researchers.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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