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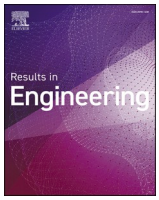

# Microwave-assisted extraction of phenolic compounds from *Commiphora gileadensis* leaf and their characterization

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ABSTRACT

*Commiphora gileadensis* (*C. gileadensis*) is a plant traditionally used in many parts of the world for medicinal purposes. However, the benefits of this plant are yet to be uncovered due to the use of conventional extraction methods during its extraction. Hence, there is a need for more efficient and environment-friendly extraction methods for optimum recovery of the bioactive components of *C. gileadensis*. This study aims to evaluate the impact of microwave-assisted extraction (MAE) process parameters (individually and in combination) on the recovery of phenolic compounds from *C. gileadensis* leaf. One-Factor-At-a-Time (OFAT) optimization method was used in this work to study the impact of varying the MAE process parameters (sample: solvent ratio, microwave power, ethanol concentration, and extraction temperature) on the optimum yield of phenolic compounds. The obtained phenolic compounds were characterized using Gas chromatography-mass spectrometry (GC–MS) for tentative identification of the component phytochemicals of the extract. The results showed that the optimal process condition of microwave power at 300 W, solvent/sample ratio of 1:10 g/mL, solvent concentration of 40 % v/v, and extraction temperature of 40 °C gave the maximum extraction yield of 33.20 ± 0.42 % w/w, total phenolic content (TPC) of 114.65 ± 3.14 mg GAE/g d.w., and total flavonoids content (TFC) of 37.56 mg QE/g d. w.). Furthermore, the GC-MS analysis identified 25 phenolic compounds with good antioxidant activities from the extracts. Therefore, MAE is considered a non-conventional green method for improved extraction of phenolic compounds from *C. gileadensis* leaf compared to the existing conventional extraction methods.

## 1. Introduction

The World Health Organization (WHO) reported that herbal products are currently serving about 80 % of the global population in numerous ways, either as food supplements or as an alternative therapy for different ailments [1,2]. Medicinal plants have been widely used since prehistoric times to treat and prevent diverse ailments and diseases. Numerous plant species have been identified for different medicinal purposes and many are yet to be discovered, hence, the recent efforts toward the diversification of alternative sources of therapeutic agents by the research community [3].

*C. gileadensis* is a 1–3 m long tree that belongs to the Burseraceae family [4–7]; it originated from the southern Kingdom of Sheba in the Arabian Peninsula [8–11] but has recently been found in other regions of the world, such as Yemen, Oman, Somalia, Ethiopia, and Sudan [12, 13]. *C. gileadensis*, also called *balsam*, is well recognized for the pricey perfume it produces, as well as the amazing health benefits of its seeds, bark, sap, wood, and leaves [14,15]. In the Middle East, the aromatic *C. gileadensis* plant is also known as *besham* or *becham* and is used in herbal medicine [4]. Since ancient times, *C. gileadensis* has served as an alternative therapy for a wide range of ailments in many Middle Eastern nations and it is still in use to date [16]. *C. gileadensis* extracts are used to manage a variety of ailments, such as stomach problems, liver problems, urinary retention, constipation, headache, and jaundice [4]. Previous studies on the phytochemical content of the parts of *C. gileadensis* revealed the presence of many phytochemical groups, such as phenolic, flavonoids, saponins, sterols, and triterpenes which suggests the suitability of the plant for both aromatic and medicinal purposes [17–19].

Various extraction methods, both conventional and non-conventional, have been used to extract bioactive compounds from

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plant matrices. A variety of techniques and solvents can be used to extract phenolics from plant materials, depending largely on their nature and distribution within the plant samples. Researchers have recently become interested in MAE, a non-unconventional method, because of its quick extraction time, higher yield quality, and little solvent use [20–23]. Microwave-assisted extraction (MAE) is one of the modern methods used to extract phenolics from plant materials [24]. It is often used because, when compared to traditional methods, it produces large amounts of phenolic compounds in a shorter period with less solvent use [25–30]. The MAE technique is essential for enhancing the extraction of phenolic compounds from plant materials with minimum inputs, especially considering energy input and environmental impacts. Through the MAE process, microwave radiation can penetrate plant materials and interact with water-soluble components to produce heat [31–33]. The effect of concentration and temperature variations in opposing directions contributes to the speed of MAE extraction operations [34,35]. The combined effect of heating and microwave power can be controlled at different or constant temperatures to achieve a successful MAE extraction technique [36,37]. During the MAE process, the pressure differential between the internal and external plant cell matrixes makes it simpler for bioactive compounds to be extruded into the solvent around them, which produces an efficient mass transfer coefficient.

To date, most research that examined the recovery yields of phenolic compounds extracted from *C. gileadensis* employed the conventional extraction techniques which are considered inefficient in the extraction of phytochemicals from plant materials; for instance, a study on the use of the solvent extraction method to extract phytochemicals from the leaf of *C. gileadensis* reported a total phenolic content (TPC) of 23.54 μg GA/mg dry weight and total flavonoids content (TFC) of 1.67 μg R/mg dry weight [38] which are considered not satisfactory. Another study found that the use of 80 % methanol during a solvent extraction process yielded a TPC of 20.97 mg GAE/g and a TFC of 6.90 mg CE/g from *C. gileadensis* leaves [38]. Additionally, the use of the maceration method has been reported to recover only 20.970 mg GAE/g and 6.90 mg GAE/g of TPC and TFC respectively, from *C. gileadensis* leaf [38].

Even though the literature demonstrated how to extract the phytoconstituents from *C. gileadensis* leaves using traditional extraction methods, the recovery yields that were achieved are too low because the right extraction method and combination of extraction variables have not been determined and used. Therefore, the major aim of this work is to efficiently extract phytochemicals from *C. gileadensis* leaf using a conventional method (MAE) which is considered effective and green. The influence of varying MAE process parameters (extraction temperature, microwave power, sample-to-solvent ratio, and solvent-concentration) on the recovery of phytochemicals from C. gileadensis leaf is also studied using the One-Factor-At-a-Time (OFAT) technique. Finally, the extracted compounds from the *C. gileadensis* leaf were identified using gas chromatography-mass spectrometry (GC-MS).

## 2. Materials and methods

### 2.1. Chemicals and reagents

Analytical grade methanol (99.9 wt %), ethanol (99.5 wt %), gallic acid, Folin-Ciocalteu phenol reagent, Quercetin, sodium carbonate anhydrous ($Na_2CO_3$), and aluminum chloride salt ($AlCl_3$) were procured "from Sigma Aldrich Sdn Bhd (Selangor, Malaysia). Distilled water was sourced from the analytical laboratory of the Faculty of Chemical and Process Engineering" Technology (FTKKP), Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA).

### 2.2. Plant material

Fresh *C. gileadensis* leaves were obtained from Hadhramout, Yemen between October to December 2021, then cleaned and dried to a stable weight in an air oven for one day at 50 °C. The dried plant material was sieved, pulverized in a grinder (RETSCH - PM 100), and stored at 4 °C.

### 2.3. Apparatus and instruments

The apparatus and instruments were used in this work were as follows. The sample was pulverized using a grinder (RETSCH - PM 100). Then, an ETHOS-microwave extractor (ATC-300, North America) was used for the extraction process (MAE). Also, the rotary evaporator (Buchi-R-200, Germany) was used to remove the solvent from the extract. Moreover, the UV–vis Spectrophotometer (Hitachi U-1800, Japan) was utilized at various wavelengths to determine the TPC and TFC absorbance. Finally, the TRACE GC ultra-system from Thermo Fisher Scientific, Waltham, Massachusetts, USA, was used to identify and quantify the components of the extract.

### 2.4. Extraction process

For the extraction process, "an ETHOS-microwave extractor (ATC-300, North America) was employed because of the ease of its control. The extraction parameters of the microwave system were managed and controlled using a programmable auxiliary input system. The system has a temperature-controlled optical fiber with a 1000 W at 1 atm maximum output power. The intake and exhaust ports of the cooling system are responsible for maintaining a boiling temperature balance and microwave power level. The following parameters were examined to analyze the effects of different MAE process variables: irradiation power (200, 300, 400, 500, and 600 W), solvent/sample ratio (1:8, 1:10, 1:12, 1:14, and 1:16 g/mL), ethanol concentration (20, 40, 60, 80 and 100 %), and extraction temperature (20, 30, 40, 50, 60, and 70 °C). The experimental scheme of the extraction procedure of extracts from *C. gileadensis* leaf using MAE can be seen clearly in Fig. 1. In a conical flask (250 mL), 10 g of the powdered plant leaf was combined with the necessary amount of ethanol: water mixture depending on the solvent/sample ratio, with the other process parameters fixed at the pre-determined level. Only one of each of the process parameters under consideration was changed at a time, with the other parameters remaining fixed at a predetermined level. After being filtered through "Whatman No.1" filter paper after each step, the extract was dried using a rotary evaporator (Buchi-R-200, Germany). The yield, TPC, and TFC of the extract were calculated after each experimental run. The experiment was carried out in triplicate and the outcomes of the extractions were expressed" as mean ± SD of three different sets of experiment.

#### 2.4.1. Extraction yields ($Y_{EX}$)

The yields of extracts from *C. gileadensis* leaves were determined and presented in dry weight (d.w.); the yields were evaluated using Eq. (1) [39].

$$Yield_{Extract} = \frac{Weight\ of\ extract\ from\ plant\ sample\ (g)}{Weight\ of\ dried\ plant\ powder\ (g)} \times 100\% \quad (1)$$

#### 2.4.2. TPC evaluation

The Folin-Ciocalteu (FC) colorimetric assay, which was previously described by Ref. [39], was used to determine the TPC with minor modifications; 10 mg of the dried extract was reconstituted in 2 mL of aqueous ethanol, and 1 mL of the reconstituted extract was mixed with 0.1 mL of FC reagent and left at room temperature for 5 min. Then, 0.5 mL of $Na_2CO_3$ solution was added to the mixture; the mixture was further allowed for 20 min before the absorbance was measured at 750 nm against the blank (pure ethanol) using "UV–vis Spectrophotometer (Hitachi U-1800, Japan)". The TPC concentration of the plant extract (which varied between 50 and 500 mg/L) was estimated using the equation line "[$y = 0.0002x + 0.0218$, $R^2 = 0.9945$]" (where; $x$: the calibration curve's sample concentration, and $y$: the absorbance at 750 nm). The tests were performed 3 times and the mean ± SD of the

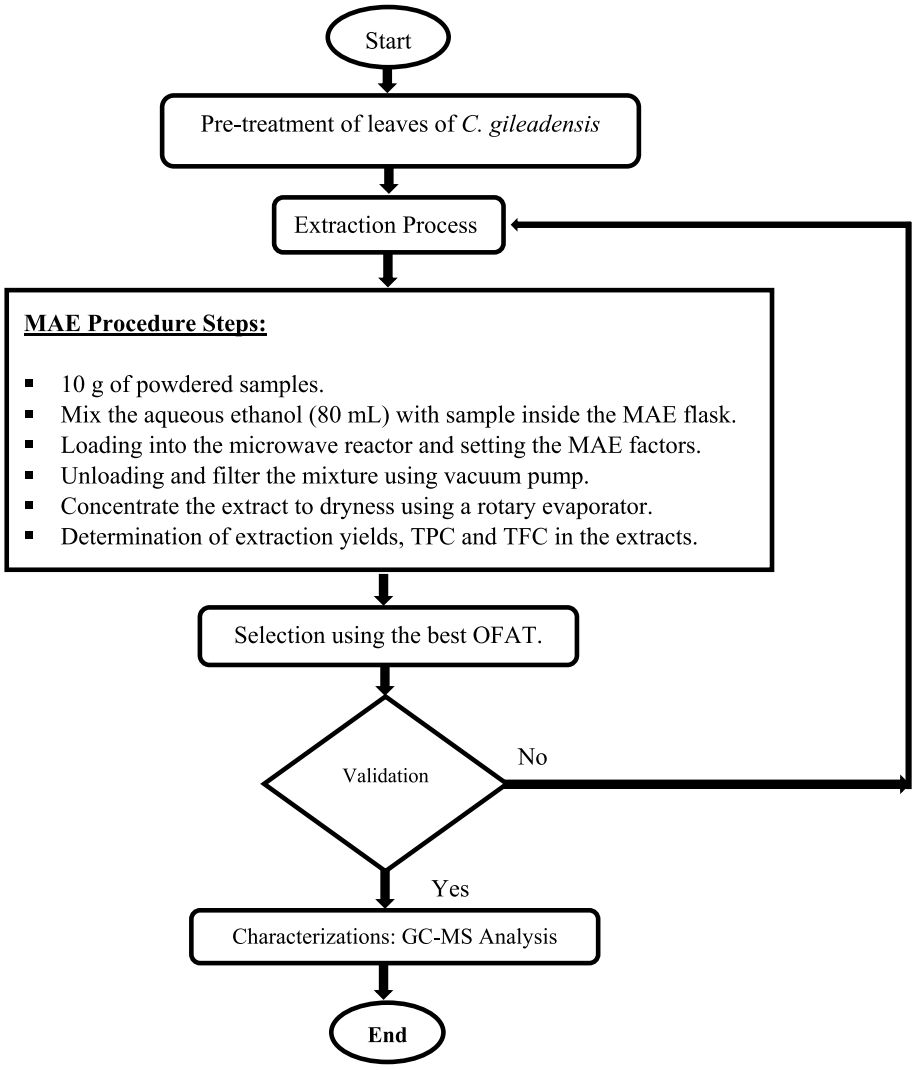


**Fig. 1.** Experiment scheme of extraction procedure of extracts from *C. gileadensis* leaf using MAE.

respective results was reported as "milligrams of gallic acid equivalents per gram sample dried weight (mg GAE/g d.w.)"." The TPC of the extract was determined as follows:

$$\mathrm{TPC} = \frac{c \times V}{m} \tag{2}$$

where c = sample concentration (mg/L), V = extraction solvent volume (L), m = dried sample weight used (g).

### 2.4.3. TFC evaluation

The TFC of the sample was assessed using a previous technique used by Ref. [39] with some modifications. A stock solution with a concentration of 1 g/L was made by dissolving 10 mg of the powdered extract in 10 mL of ethanol; aluminum chloride solution was prepared from 2 g of $AlCl_3$ and 100 mL of ethanol; then, 1 mL of $AlCl_3$ solution was combined with the extract (1 mL). The absorbance of this mixture was measured at 420 nm using "UV–vis Spectrophotometer (Hitachi U-1800, Japan)" after the mixture had been left to thoroughly react at room temperature for 1 h (h). Following that, the TFC concentration of the plant extract (which varied between 50 and 500 mg/L) was estimated using the equation line "[$y = 0.0023x + 0.0374$, $R^2 = 0.9963$]" (where $x$: the sample concentration from the calibration curve, and $y$: the absorbance at 420 nm). The TFC of the extract was evaluated using Eq. (3). The tests were done in triplicates and the result was reported as the mean ± SD of the respective results. The TFC was represented as "milligrams of quercetin equivalents per gram dried sample weight (mg QE/g d.w.)"."

$$\mathrm{TFC} = \frac{c \times V}{m} \tag{3}$$

where $c$ = sample concentration (mg/L), $V$ = sample volume (L), $m$ = dried sample weight used (g).

## 2.5. Characterization studies

### 2.5.1. GC-MS analysis

The obtained leaf extract of *C. gileadensis* at the optimized condition was subjected to GC-MS analysis to identify and quantify the components as described by Ref. [17] with some modifications. A TRACE GC ultra-system from Thermo Fisher Scientific, Waltham, Massachusetts, USA, fitted with a 30 m × 0.25 mm x 0.25 m Elite-5-MS capillary column was used to analyze the extracts. The temperature of the column during the analysis was raised from 40 °C to 220 °C at a rate of 4 °C/min. The injection volume of 1 μL was maintained at the injector temperature of 250 °C; helium gas served as the carrier gas at the flow rate of 20 mL/min; the transfer temperature was maintained at 280 °C. The following MS settings were used: EI mode, 70 eV for the ionization voltage, 180 °C for the ion source temperature, and a scan range of 50–600 Da. Tentative identification of the peaks was achieved based on

a library search using NIST and Wiley Registry 8th Edition.

### 2.6. Statistical analysis

MS Excel was used to calculate the average of the triplicated experiments for the statistical analyses, which were conducted using one-way ANOVA ($P < 0.05$). The precision of the procedure was confirmed between three replicated trials using the ANOVA ($P < 0.05$) analysis. Each factor (extraction time, solvent concentration, and sample/solvent ratio) was evaluated for the calculated values of the responses, extraction yield, TPC, and TFC. The standard deviation between the three means was also computed, and the results were reported as mean ± SD. Furthermore, using a student t-test, the results from all three replicated studies were examined for significant differences at $P < 0.05$.

## 3. Results and discussion

### 3.1. Effect of microwave power on the recovery yields

The major feature that distinguishes MAE from other methods is microwave power; the effect of this process variable on the rate of TPC yield from the leaves of *C. gileadensis* was determined in this study. Microwave power (MW power) is characterized by the generation of "localized heating, adsorption, and distribution of energy from the extraction solvent to the sample, which results in the breaking of the cell wall and the ejection of the bioactive compounds [32]. As per reports, there is a connection between microwave and temperature since an increase in microwave power raises the temperature, improving yields [32,34]. The impacts of microwave power were studied at various values at 200, 300, 400, 500, and 600 W in the MAE process at a constant sample/solvent ratio of 1:08 g/mL, 20 % v/v ethanol concentration, and 20 °C of extraction temperature. According to Fig. 2, the TPC and TFC rapidly increased with the MW power during the washing phase and continued to rise during the diffusion phase. But, as the driving forces increased, the yields continued to improve as the microwave power changed between 200 and 300 W. More increase in microwave power (>300 W) resulted in a decrease in the recovery yields; extraction yield, TPC, and TFC, probably due to the excessive heating caused by high microwave power might degrade heat-sensitive phenolic and flavonoid components and reduce extraction efficiency. Higher power levels can also result in hot patches that can further degrade target compounds or evaporate the solvent too soon, which lowers the overall recovery yield. Higher power levels can also cause non-uniform temperature distributions inside the extraction matrix. The ideal microwave power guarantees adequate energy for effective extraction without jeopardizing the integrity of the bioactive chemicals. Power levels above 300 W frequently transcend this threshold, resulting in the yield drop that has been observed. This showed that 300 W was the ideal MW power setting for efficient extraction of most phenolic compounds from *C. gileadensis* leaf using MAE. At the optimal condition, the percentage extract yield was 30.5 ± 0.46 w/w%, while TPC and TFC yields were 83.41 ± 2.37 mg GAE/g d.w. and 27.35 ± 1.56 mg QE/g d.w, respectively. This result is similar to previous research that examined the extraction of pectin from industrial tomato waste using MAE, where the optimal microwave power was 300 W to achieve a high pectin yield with a higher galacturonic acid and lycopene content [40]. Furthermore, as previously indicated, the MP of 300 W produced the maximum recovery yields in this study (Fig. 2). The statistical significance of this finding was verified using one-way ANOVA, which suggests that 300 W MP is more effective than the other tested MP levels. The significance of this finding was also validated by examining the significant differences in the results using a student t-test ($P < 0.05$). As a result, the ideal level of microwave power in this study was 300 W, which was employed in the rest of the investigation.

### 3.2. Effect of solvent/sample ratio on the recovery yields

A higher amount of solvent may favor and boost the mass transfer rate, according to studies; however, using too much solvent may demand additional energy. Thus, it is crucial to determine the exact quantity of solvent required when extracting bioactive compounds from plant material. The findings regarding the impacts of various sample/solvent ratios on the yields, TPC, and TFC of *C. gileadensis* leaf using the MAE method are shown in Fig. 3. At a fixed MW power of 300 W, 20 % v/v ethanol concentration, and 20 °C temperature, the impact of the ratio of the sample to solvent on the process was investigated at different ranges (1:08, 1:10, 1:12, 1:14, and 1:16 g/mL). There were significant increases in "the yield, TPC, and TFC of the extract during the washing and diffusion phases. However, the maximum yield was observed at the sample/solvent ratio of 1:10 g/mL as" further increases beyond this ratio negatively affected the yields. This might be due to the extra time and energy needed to reach the equilibrium yield, TPC, and TFC in a larger solvent volume. Additionally, at higher sample/solvent ratios, the solute concentration was reduced, which increased the driving power for diffusion and dissolution. This is not the case at lower solute concentrations where the driving force for adsorption will be diminished [41]. As a result, a sample/solvent ratio of 1:10 g/mL was determined as the

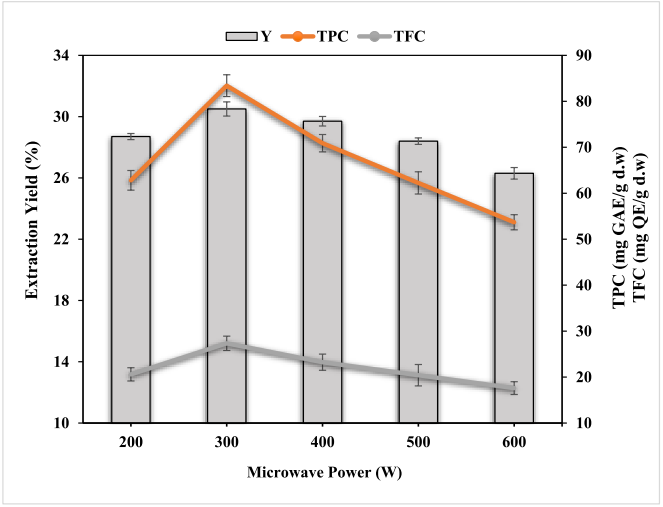


**Fig. 2.** Effects of microwave power (W) on the recovery yield, TPC, and TFC of *C. gileadensis* leaf using MAE.

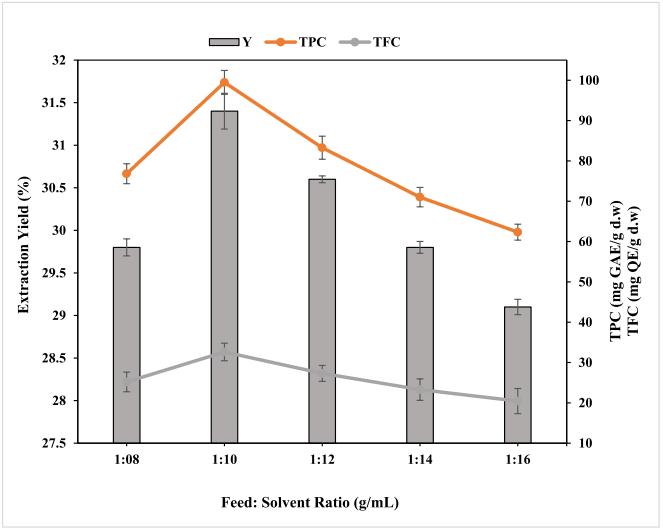


**Fig. 3.** Effects of feed-to-solvent ratio (g/mL) on the recovery yield, TPC, and TFC of *C. gileadensis* leaf using MAE.

ideal ratio for optimal recovery of bioactive components from the leaf of *C. gileadensis* using MAE. The achieved TFC, TPC, and extraction yield were "32.62 ± 3.04 mg QE/g d.w, 99.48 ± 3.09 mg GAE/g d.w., and 31.40 ± 0.25 w/w%", respectively. This outcome is consistent with the previous findings on the extraction of total phenolic compounds from *Eleutherine bulbosa (Mill.) urb*. using the MAE technique at the ideal sample/solvent ratio of 1:10 g/mL [42]. Similarly, a sample/solvent ratio of 1:10 g/mL produced the best phytochemical yields. A one-way ANOVA and a student t-test were used to validate that this sample ratio is statistically more contributory to the yield of phytochemicals from the studied plant material using the MAE method ($P < 0.05$). Therefore, the 1:10 sample/solvent ratio was selected for the subsequent studies.

### 3.3. Effect of ethanol concentration on the recovery yields

Ethanol, which is widely the solvent of choice for the extraction of phenolic compounds from plant materials, is a low-toxicity polar green solvent that is soluble in water at any concentration [39]. The impacts of ethanol concentrations (20, 40, 60, 80, and 100 % v/v) on the yield, TPC, and TFC of the sample at a fixed microwave power of 300 W, a sample-to-solvent ratio of 1:10 g/mL, and process temperature of 20 °C is depicted in Fig. 4. The yields, TPC and TFC increased as the ethanol concentration rose from 20 to 40 % v/v, but after 40 % v/v, further increases caused the yields to gradually decline. A few drops of water may hasten the mass transfer process by increasing the solvent's relative polarity, which enhances the solvent's capacity to dissolve organic molecules by expanding the plant matrix [39]. Therefore, 40 % v/v ethanol concentration was selected as the best for optimal recovery of bioactive components from the leaf of *C. gileadensis* using MAE. Furthermore, it was discovered that an ethanol concentration of 40 % v/v gave the optimum yield of phytochemicals (32.50 ± 0.31 % w/w), TPC yield of 109.11 ± 2.17 mg GAE/g d.w., and TFC yield of 35.77 ± 3.14 mg QE/g d.w. A one-way ANOVA and a student t-test were used to validate that ethanol concentration of 40 % v/v is statistically more contributory to the yield of phytochemicals from the studied plant material using the MAE method ($P < 0.05$). These outcomes are in line with previous research that found the best yields of phenolic compounds from *Myrtus communis* L. leaves using 40 % v/v ethanol concentration [43]. Thus, for the subsequent investigation (extraction temperature), the ethanol concentration of 40 % v/v was maintained.

### 3.4. Effect of microwave temperature on the recovery yields

Process temperature during MAE is a critical factor that influences the leaching of phenolic compounds from plant materials. Temperature increases can make it easier for the solvent to penetrate the core of the plant material, producing more extracts. The Einstein equation states that higher temperatures result in increased diffusion rates due to the related reductions in solvent viscosity [44]. Despite this, phenolic compounds can deteriorate if exposed to higher temperatures over an extended period [37]. Fig. 5 showed that temperatures between 20 and 40 °C were the best for the extraction of phenolic compounds from *C. gileadensis* leaves using MAE as the optimum yield was observed within this temperature range. However, the washing phase witnessed a rapid increase in the TPC and TFC which gradually improved throughout the diffusion phase. Furthermore, a gradual increase in the temperature (from 20 to 40 °C) steadily improved the TPC and TFC but above 40 °C, minor declines were noticed in the TPC and TFC. Mild heating has been shown to weaken cell walls and accelerate the ejection of trapped phenolics [45]. If the temperature is not high, it generally has a favorable impact on the speed and effectiveness of extraction operations; nevertheless, when the temperature is extreme, bioactive chemicals can be destroyed [46]. Furthermore, the high rates of solvent losses during MAE processes at high temperatures could lead to the loss of volatile compounds. Consequently, 40 °C was chosen as the ideal temperature for optimal recovery of bioactive components from the leaf of *C. gileadensis* using MAE. This is in line with earlier studies that demonstrated a temperature of 40 °C as the optimal microwave temperature for phycoerythrin (PE) extraction from phycobiliproteins of *Porphyridium purpureum* (*Pp*) [47]. At a fixed microwave power of 300 W, sample/solvent ratio of 1:10 g/mL, ethanol concentration of 40 % v/v, and microwave temperature of 40 °C, the yield, TPC, and TFC of the leaf extract were "33.20 ± 0.42 % w/w, 114.65 ± 3.14 mg GAE/g d.w. and 37.56 ± 1.76 mg QE/g d.w.", respectively. This result is a significant improvement compared to the recent reports on the extraction of bioactive compounds from *C. gileadensis* leaf using other methods such as solvent extraction, where the optimal TPC and TFC were reported as "20.97 mg GAE/g d.w. and 6.90 mg QE/g d.w., respectively" [17,48]. Besides, 40 °C was the ideal microwave temperature for the maximal recovery of phytochemicals from *C. gileadensis* leaf. A one-way ANOVA and a student t-test were used to validate that a temperature of 40 °C is statistically more contributory to the yield of phytochemicals from the studied plant material using the MAE method ($P < 0.05$).

Overall, the results obtained via MAE were about 4 times higher than

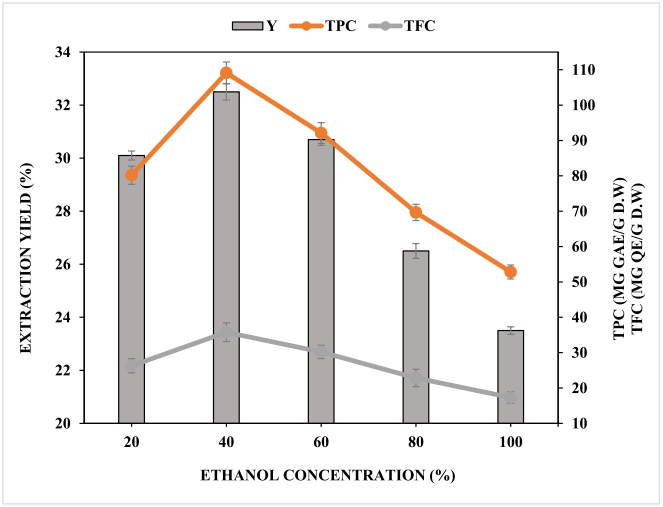


**Fig. 4.** Effects of ethanol concentration (% v/v) on the recovery yield, TPC, and TFC of *C. gileadensis* leaf using MAE.

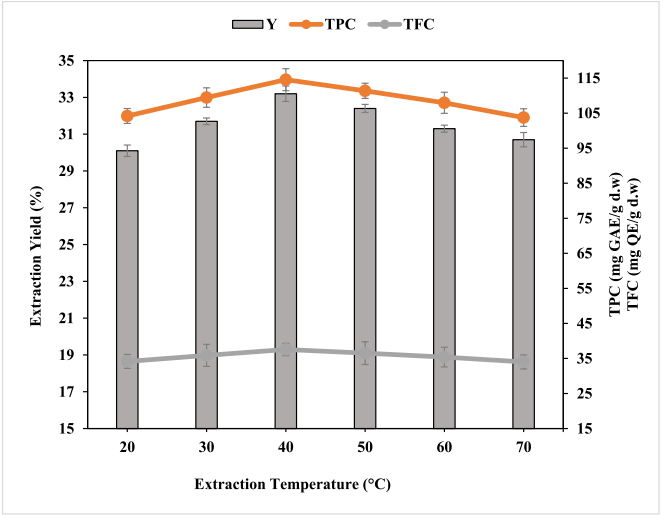


**Fig. 5.** Effects of temperature (°C) on the recovery yield, TPC, and TFC of *C. gileadensis* leaf using MAE.

the 20.97 mg GAE/g d.w for TPC and 6.90 mg QE/g d.w for TFC obtained by previous scholars from the same plant material [17,49,50] using maceration and solvent extraction methods, respectively. This reflected the advantage of using MAE over the conventional methods as it required shorter extraction time and reduced solvent to achieve better TFC and TPC yields from the leaf of *C. gileadensis*.

### 3.5. Characterization

The *C. gileadensis* leaf extracts were obtained at the optimal MAE condition of microwave power 300 W, sample/solvent ratio 1:10 g/mL, ethanol concentration 40 % v/v, and microwave temperature 40 °C; the extracts were further characterized using GC-MS (for tentative identification of the phytochemicals).

The chemical components of the *C. gileadensis* leaf in this study (extracted using MAE) were identified by GC-MS analysis as shown in Fig. 6 and Table 1. Generally, 25 phytochemicals were tentatively identified in the *C. gileadensis* leaf extract as represented by their respective peaks. The presence and relative quantity of various chemicals are shown by the peaks in the GC-MS chromatogram (Fig. 6). Each peak's height corresponds to the extract's associated compound's concentration. The presence of chemicals in the extract at substantially greater quantities is indicated by high peaks in the GC-MS chromatogram. Such compounds with high peaks often have a bigger impact on the extract's overall chemical profile and biological activity; for example, the present research revealed multiple conspicuous peaks that are associated with significant bioactive chemicals, including phenol, cyclooctasiloxane, cycloheptasiloxane, heptasiloxane, and other derivatives of siloxane. The existence of these chemicals in high concentrations indicates that the MAE process is successful in concentrating these bioactive elements, which have been linked to possible medicinal and antioxidant benefits [51,52]. On the other hand, low peaks indicate the presence of chemicals at lower quantities; these substances may add to the extract's overall complexity and efficacy even if when in low concentration via synergistic or antagonistic associations [53].

The tentatively identified 25 phytochemicals in the *C. gileadensis* leaf extract belong to the hydrocarbons, alcohols, esters, and fatty acids groups. Among these compounds, the most abundant phytochemicals are: Phenol and 2,4-bis(1,1-dimethylethyl)-(5.97 %) which have been reported to exhibit anti-inflammatory, antioxidant, insecticidal, cytotoxic, antiviral, nematicidal, phytotoxic, antifungal, and antibacterial activities [54], Cycloheptasiloxane and tetradecamethyl-(8.05 %) are reported to have antimicrobial and anticancer activity [55]; Cyclodecasiloxane and eicosamethyl-(11.72 %) have been shown to have hepato-protective, anti-rheumatic, and anti-spasmodic properties [56]; Cyclononasiloxane and octadecamethyl-(12.02 %) are anti-fungal agents [57]; Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-(12.85 %), Cyclooctasiloxane, and hexadecamethyl-(12.94 %) have proven antimicrobial, and antioxidant activities [58,59]; Heptasiloxane and hexadecamethyl-(13.54 %) are reported to have antifungal, anti-inflammatory, antiarthritic, antimicrobial, antioxidant, antiasthma, diuretic, and analgesic properties [60]. It is interesting to note that some of these phytochemicals had earlier been reported in bark extracts of *C. gileadensis* but at lower concentrations [17] compared to their observed concentration in this study; this variation in concentration of these phytochemicals could be due to the type of extraction method used, as well as the geographical location of the plant material. Further, it is interesting that these phytochemicals from *C. gileadensis* leaf extracts obtained through MAE contained a diverse set of bioactive phytoconstituents with potent antioxidant activity.

## 4. Conclusion

The use of MAE as an environment-friendly method for the extraction of phenolic compounds from C. *gileadensis* leaf was reported in this study. The study aims to study the influence of varying the MAE process parameters (extraction temperature, microwave power, sample-to-solvent ratio, and solvent-concentration) on the yield of phenolic compounds from the leaves of *C. gileadensis,* and to reach this objective, the 'One-Factor-At-a-Time' (OFAT) technique was employed. The obtained extracts were characterized for phytochemical constituents using GC-MS. The results showed that the maximum phytochemical recovery yields of 33.20 ± 0.42 %, TPC of 114.65 ± 3.14 mg GAE/g d.w, and TFC of 37.56 ± 1.76 mg QE/g d.w were obtained from the leaves of *C. gileadensis* at the MAE process condition of microwave power at 300

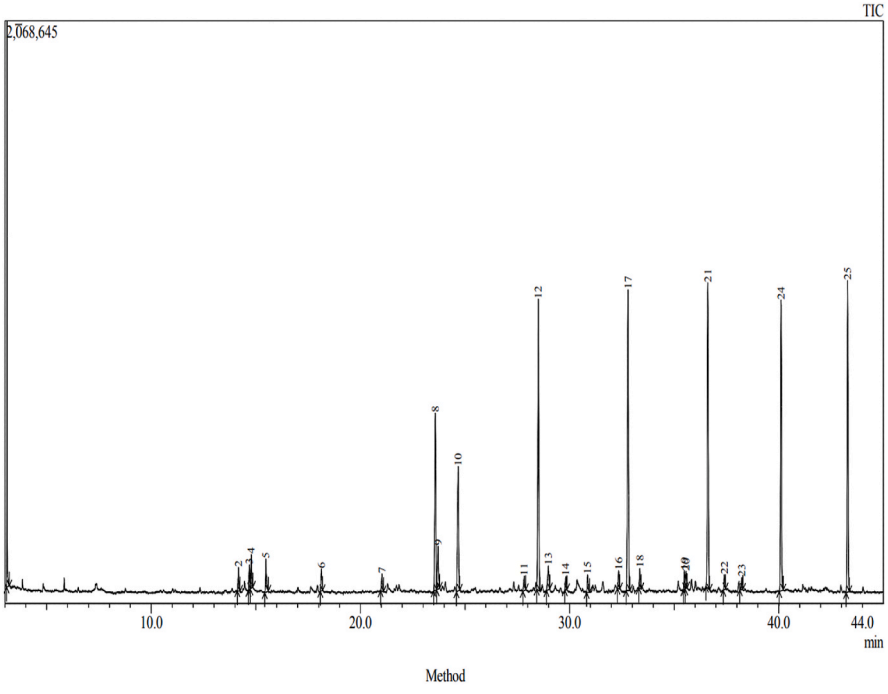


**Fig. 6.** GC-MS analysis of *C. gileadensis* leaves extract from the MAE process.

**Table 1**
GC-MS analysis of *C. gileadensis* leaf extract.

| Peak | Compound Name | Peak Area (%) | Mol. Formula | Mol. Weight | R.T (min) |
|---|---|---|---|---|---|
| 1. | Silane, triethylfuoro- | 7.55 | $C_6H_{15}FSi$ | 134 | 3.100 |
| 2. | Cycliopropane, nonyl | 0.89 | $C_{12}H_{24}$ | 168 | 14.170 |
| 3. | 1-Diisopropylsilyloxycyclohexane | 0.82 | $C_{12}H_{26}OSi$ | 214 | 14.685 |
| 4. | Butanenitrile, 3-chloro-3-methyl- | 1.20 | $C_5H_8C1N$ | 117 | 14.775 |
| 5. | 1-propanone, 2-bromo-1-phenyl- | 1.44 | $C_9H_9BrO$ | 212 | 15.480 |
| 6. | Cyclohexanesiloxane, dodecamehyl- | 0.96 | $C_{12}H_{36}O_6Si_6$ | 444 | 18.125 |
| 7. | 5-Octadecane, (E)- | 0.79 | $C_{18}H_{36}$ | 252 | 21.025 |
| 8. | Cycloheptasiloxane, tetradecamethyl- | 8.05 | $C_{14}H_{42}O_7Si_7$ | 418 | 23.580 |
| 9. | Decyl trifluoroacetate | 1.88 | $C_{12}H_{21}F_3O_2$ | 254 | 23.710 |
| 10. | Phenol, 2,4-bis(1,1-dimethylethyl)- | 5.97 | $C_{14}H_{22}O$ | 206 | 24.670 |
| 11. | Lauryl acetate | 0.69 | $C_{14}H_{28}O_2$ | 228 | 27.830 |
| 12. | Cyclooctasiloxane, hexadecamethyl- | 12.94 | $C_{16}H_{48}O_8Si_8$ | 592 | 28.505 |
| 13. | 2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-tri,ethyl-8-methylene- | 1.32 | $C_{15}H_{26}O$ | 222 | 28.980 |
| 14. | 1-Tetradecanol | 0.54 | $C_{14}H_3O$ | 214 | 29.815 |
| 15. | Ethanol, 2-(dodecyloxy)- | 0.83 | $C_{14}H_{30}O_2$ | 230 | 30.855 |
| 16. | Butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)- | 0.70 | $C_{34}H_{34}$ | 442 | 32.345 |
| 17. | Heptasiloxane, hexadecamethyl- | 13.54 | $C_{16}H_{48}O_6Si_7$ | 532 | 32.795 |
| 18. | Triazine, 2-amino-4-(piperidino, ethyl)-4-piperidino- | 0.76 | $C_{14}H_{24}N_6$ | 276 | 33.355 |
| 19. | Succinic acid, di(4-isopropylphenol) ester | 0.75 | $C_{22}H_{26}O_4$ | 354 | |
| 20. | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- | 12.85 | $C_{16}H_{50}O_7Si_8$ | 578 | |
| 21. | 1-(+)-Ascrobic acid 2,6-dihexandecanonate | 0.57 | $C_{38}H_{68}O_8$ | 652 | 37.395 |
| 22. | Hexadecenoic acid, ethyl easter | 0.52 | $C_{18}H_{36}O_2$ | 284 | 38.230 |
| 23. | Cyclodecasiloxane, eicosamethyl- | 11.72 | $C_{20}H_{60}O_{10}Si_{10}$ | 720 | 40.110 |
| 24. | Cyclononasiloxane, octadecamethyl- | 12.02 | $C_{18}H_{54}O_9Si_9$ | 666 | 43.295 |

W, sample: solvent ratio of 1:10 g/mL, solvent concentration of 40 % v/v, and microwave temperature of 40 °C. The GC-MS analysis tentatively identified a total of 25 chemical compounds in the extract compared to the 19 compounds earlier reported in the literature using conventional methods such as solvent extraction. Thus, the MAE method is considered an ideal green extraction method for better extraction of phenolic compounds from the leaf of *C. gileadensis*. More so, further optimization studies of the MAE process using two-level-factorial design and central composite designs could improve knowledge on the interaction of process parameters during the MAE process for better experimental designs and improved recovery of phytochemicals from the leaf of *C. gileadensis*.

## CRediT authorship contribution statement

**Aiman A. Bin Mokaizh:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Abdurahman Hamid Nour:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Chinonso Ishmael Ukaegbu:** Writing – review & editing, Writing – original draft.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that has been used is confidential.

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