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Extraction and Characterization of Biological Phytoconstituents of *Commiphora gileadensis* Leaves Using Soxhlet Method

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Abstract: *Commiphora gileadensis* is a medicinal plant with a wide range of biological characteristics. Many medical diseases can be treated using the leaves of *C. gileadensis*, including bacterial infections, inflammatory illnesses, and wounds. As a result, the Soxhlet extraction method was used to extract the phenolic components and measure the recovery yields from *C. gileadensis* leaf. The impacts of the Soxhlet extraction parameters (extraction time 30–150 min, sample/solvent ratio 1:20–1:40 g/mL, and concentration of ethanol solvent 20–100% *v*/*v*) on the total flavonoid content (TFC), total phenolic content (TPC), and extraction yield were investigated using the one-factor-at-a-time (OFAT) technique. Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectroscopy (GC–MS) analyses have been employed to evaluate the extracts for the presence of various phytochemicals. According to the results, the *C. gileadensis* leafs ethanolic extract extracted via the Soxhlet process achieved the maximum yields at 90 min of extraction time, a feed/solvent ratio of 1:30 g/mL, and a 40% *v*/*v* ethanol concentration. These yields were: extraction yield = 23.20 ± 0.10% *w*/*w*, TPC = 59.93 ± 1.33 mg GAE/g d.w., and TFC = 19.65 ± 1.77 mg QE/g d.w.. Further, a total of 20 phenolic components with excellent antioxidant characteristics were found in the leaf extract of *C. gileadensis* extracted via the Soxhlet method.

Keywords: extraction process; total phenolic content; *C. gileadensis* leaf; total flavonoid content

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1. Introduction

Since ancient times, plants for medicinal purposes have been historically utilized to cure and prevent a wide range of diseases and problems. Recently, developing nations all over the world have started to believe in the efficacy, safety, and full protection of herbal treatments and medicinal plants [1]. As reported by the World Health Organization (WHO), 80% of people utilize herbal products nowadays in a variety of ways, including as dietary supplements or as an alternative treatment for a number of disorders [2,3]. Despite the fact that many plants are already used as sources of complementary medicine, many more are still to be explored that must be discovered in order to assure sustainability [4].

Commiphora gileadensis is a one-to-three-meter-tall tree belonging to the Burseraceae family [5–9]. It originated from the southern Kingdom of Sheba in the Arabian Peninsula [10–13]. In recent times, other parts of the world, including Yemen, Oman, Somalia, Ethiopia, and Sudan, have discovered it [14–16]. As per [15,17], the plant *C. gileadensis*,

popularly known as balsam, is well-renowned for both the pricey fragrance it generates and the extraordinary health benefits of its seeds, bark, sap, wood, and leaves. In the Middle East, the aromatic *C. gileadensis*, sometimes known as besham or becham, has been used to manufacture herbal remedies as an alternative cure for a variety of illnesses since antiquity, and it is still in use today [18]. In fact, *C. gileadensis* has a strong anti-cancer impact on cancer cell lines and might be effective in treating a variety of diseases [5,19]. Likewise, it offers medicinal advantages for the treatment of ailments such as jaundice, liver problems, constipation, urinary retention, and stomach issues [17].

The aerial parts of this plant were examined using phytochemical methods, and phenolics, triterpenes, flavonoids, sterols, and saponins were revealed [20,21]. The plant seems to be appropriate for both its medicinal properties and its advantageous aromatic resin [22]. Further, this plant is utilized as a diuretic, analgesic, and treatment for opportunistic infections in traditional Arabic medicine in numerous African nations [23,24].

Extraction is a key technique for recovering phenolic constituents from plant matrices. It is feasible to use both traditional and unconventional procedures [25]. For many years, conventional methods like boiling, maceration, soaking, hydro-distillation, and Soxhlet were frequently used. In contrast to other conventional methods like percolation or maceration, extraction using the Soxhlet method is thought to be the most widely used method for phenolic compound extraction since it takes less solvent and less time, is easy to use, is good for total extract recovery, is appropriate for initial and bulk extraction, and can be completed quickly [26,27]. Furthermore, Soxhlet extraction is a firmly established technique that outperforms other traditional extraction methods, particularly for thermolabile extraction chemicals [26]. Additionally, this approach can provide greater yields with far less solvent in comparison to other conventional methods [28–30].

To the best of the authors' knowledge, few studies in the literature have carried out traditional extraction methods and focused on the recovery yields of phenolic compounds extracted from *C. gileadensis*. One study used the solvent extraction method and reported the outcomes as TFC = 1.67 µg R/mg dry weight and TPC = 23.54 µg GA/mg dry weight [16]. Another study reported that the TPC was 20.97 mg GAE/g, and TFC was 6.90 mg CE/g, extracted from *C. gileadensis* leaf using 80% methanol solvent extraction [16]. Furthermore, a study recorded that the TPC and TFC of the leaf of *C. gileadensis* were 20.970 mg GAE/g and 6.90 mg GAE/g, respectively, as outcomes of using the maceration method [16]. Although the literature highlighted the usage of conventional extraction methods to extract the phytoconstituents of *C. gileadensis* leaf, the obtained recovery yields were too low due to the selection of extraction method and the lack of an appropriate combination of extraction parameters.

Therefore, the focus of this work was on the Soxhlet extraction of phenolic compounds and extraction yield extracted from the leaves of *C. gileadensis* utilizing the OFAT method. Moreover, at the maximum Soxhlet extraction conditions, Fourier transform infrared spectrometry and gas chromatography–mass spectrometry were utilized to analyze the functional groups and phenolic compounds in the extract.

2. Materials and Methods

2.1. Sample Preparation

Fresh leaves of *C. gileadensis* were collected from Hadhramout, Yemen, between October and December 2021. The sample was cleaned and dried to a stable weight in an air oven at 50 °C for one day. The dried plant material was pulverized in a grinding machine (RETSCH—PM 100), sieved, and refrigerated at 4 °C for further use.

2.2. Chemicals and Reagents

Analytical-grade methanol (99.9 wt%), ethanol (99.5 wt%), aluminum chloride salt ($AlCl_3$), gallic acid, Quercetin, Folin–Ciocalteu (FC) phenol reagent, and sodium carbonate anhydrous (Na_2CO_3) were procured from Sigma Aldrich Sdn. Bhd. The Faculty of Chemical

and Process Engineering Technology at Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA) provided the analytical lab where the distilled water was obtained.

2.3. Extraction Procedure

A 10 g powdered sample of the *C. gileadensis* leaves was placed into a thimble, which was then loaded inside the Soxhlet extractor. A 500 mL round-bottom flask with a condenser was attached to the Soxhlet extractor and filled with the ethanol–water combination. The extractor was then set up on a heating mantle. The solvent started to evaporate as it moved through the Soxhlet extractor to the condenser after being heated by the heating mantle. Next, drips of the condensed solvent started appearing in the Soxhlet extractor holding the thimble that contained the plant sample. The solvent with extract was recycled back to the round-bottom flask once the solvent level reached the siphon. This process continued until the designed period for the extraction had finished, and then the solution of the extract was given time to cool down at ambient temperature. Following that, the extract was concentrated via a rotary evaporator after being filtered using Whatman No.1 filter paper. After that, the extraction yields, TPC and TFC, were obtained. The experimental trials were carried out three times, and mean data were provided. The dried extract was then kept chilled at 4 °C for further testing.

2.4. Determination of Extraction Yields ($Y_{Extract}$)

The extraction yields were determined using Equation (1) and presented as dry weight (d.w.) [31].

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

2.5. Total Phenolic Content Determination

The Folin–Ciocalteu (FC) colorimetric test was modified slightly to determine TPC [31]. In 2 mL of aqueous ethanol, around 10 mg of the dried extract was redissolved; 0.1 mL of FC reagent was combined with 1 mL of the extract and left for 5 min at ambient temperature. Afterwards, 0.5 mL of the solution of Na_2CO_3 was poured into the mixture; this mixture was further allowed to sit for 20 min before the absorbance measurement of 750 nm against the pure ethanol (blank) via UV–Vis spectrophotometer (Hitachi U-1800, Tokyo, Japan). The TPC concentration present in the plant's extract varied between 50 and 500 mg/L. The TPC of the extract was evaluated via Equation (2) [31]. The tests were run three times, and the outcomes were given as milligrams of gallic acid equivalents per gram sample dried weight (mg GAE/g d.w.) based on the mean and ±SD of the individual results.

$$\mathrm{TPC} = \frac{c \times V}{m} \quad (2)$$

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

2.6. Total Flavonoid Content Determination

The TFC of the sample was evaluated utilizing an earlier method with some changes employed [31]. To prepare a stock solution with a concentration of 1 g/L, 10 mg of dried plant extract was diluted in 10 mL of ethanol. Then, the aluminum solution chloride 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). After 1 h of allowing the mixture to totally react at room temperature, a UV–Vis spectrophotometer (Hitachi U-1800, Japan) was used to measure the absorbance of the mixture at 420 nm. Following that, the TFC concentration present in the plant's extract varied between 50 and 500 mg/L. The TFC of the extract was evaluated using Equation (3). The tests were run three times, and the outcomes were reported as

the mean and ±SD of each set of data. The TFC was denoted as milligrams of quercetin equivalents per gram dried sample weight (mg QE/g d.w.).

$$\text{TFC} = \frac{c \times V}{m} \quad (3)$$

2.7. Fourier Transform Infrared Spectroscopy Analysis

The functional groups found in the *C. gileadensis* leaf extract were identified using Fourier transform infrared spectroscopy analysis. FTIR (Nico-let iS5 iD7 ATR; Thermo Scientific, Dortmund, Germany) fitted with OMNIC software 9.1 was utilized for this analysis. The extracts were analyzed to produce IR spectra between 500 and 4000 cm^{-1} with a resolution of 4 cm^{-1} [32]. To identify the observed peaks in the sample, they were compared to a database of anticipated bands of absorption for the various bonds and groups for the molecule.

2.8. Chromatography–Mass Spectrometer Analysis

The leaf extract of *C. gileadensis* was tested by chromatography–mass spectrometer analysis to detect and quantify the components as described by [31] with some modifications. A TRACE GC ultra-system from Thermo Fisher Scientific, Waltham, MA, USA, fitted with a 30 m × 0.25 mm × 0.25 m Elite-5-MS capillary column, was used to analyze the extracts. The temperature of the column during the analysis was raised at a rate of 4 °C/min from 40 °C to 220 °C. The injection volume of 1 µL was maintained at the injector temperature of 250 °C; the transfer temperature was maintained at 280 °C and at the flow rate of 20 mL/min, with helium as the carrier gas. The following MS settings were used: EI mode, 70 eV for the ionization voltage, a range of scan of 50–600 Da, and 180 °C for the ion source temperature. Tentative identification of the peaks was achieved based on a library search using Wiley Registry 8 Edition and NIST.

2.9. Statistics Analysis

The statistical analyses, which were carried out using one-way ANOVA ($p < 0.05$), were performed using the average of the triplicated experiments through MS Excel (https://www.microsoft.com/en/microsoft-365/excel?market=af). The ANOVA ($p < 0.05$) analysis was used to validate the method's accuracy between triplicated experiments. The calculated value of the responses—extraction yield, TPC, and TFC—was tested for each factor (extraction time, solvent concentration, and sample/solvent ratio); the standard deviation between the three means was also calculated and the results were presented as mean ± SD. The responses throughout the triplicated experiments were also tested for significant differences at $p < 0.05$ using a student *t*-test.

3. Results and Discussion

The effects of extraction parameters such as extraction time, concentration of ethanol, and feed/solvent ratio on the recoveries of TFC, TPC, and yield extracts from *C. gileadensis* leaves were investigated using the Soxhlet extraction method. Table 1 illustrates the influence of individual parameters on yields. The following is a discussion of the impact of each factor.

3.1. Extraction Time Effect

The fact that extract yields tend to rise with longer extraction times means that there is always a possibility that bioactive components might be degraded. Thus, the Soxhlet extraction's extraction time is a key factor in determining how effectively bioactive chemicals are extracted, particularly with respect to the recovered yield and the extraction of phenolic constituents [26]. Extraction time must be taken into consideration to reduce energy and cost usage. It is one of the crucial elements that affects how well phenolic chemicals are recovered from the plant matrix. The reason is that excessive local heating of plant samples tends to destroy phenolic chemicals [33]. Therefore, choosing the right

extraction time is essential for achieving an extreme recovery. Hence, the influences of varying the extraction time on the TFC, TPC, and yield of extracts were examined via a Soxhlet extraction. The experiment studied the effects of using different extraction times of 30, 60, 90, 120, and 150 min, while fixing the concentration of ethanol at 20% v/v and the ratio of the feed/solvent at 1:20 g/mL. After the extraction had been increased from 30 min to 90 min, the utmost TFC, TPC, and extraction yield were recorded (as seen in Table 1). The highest recoveries were obtained at this time (90 min) as 19.5 ± 0.15% w/w for the extract yield, 48.22 ± 1.05 mg GAE/g d.w. for TPC, and 15.81 ± 1.19 mg QE/g d.w. for TFC. However, when the extraction time increases more, the phenolic constituents recovery declines; this could be a result of deterioration brought on by warming plant material [34,35]. Also, this might be a result of the phenolic constituents degrading as a consequence of overheating the samples of plants due to longer extraction time [34]. The optimal extraction time of 90 min yielded the highest recovery yields, as mentioned above (Table 1). The statistical significance of this outcome, confirmed through one-way ANOVA, indicates that the 90 min extraction time was notably more effective than other evaluated durations. The reliability of these extraction parameters was further corroborated by employing a student *t*-test ($p < 0.05$) to examine significant differences in the responses, thereby providing a robust foundation for our study's conclusions. The outcomes were also in accordance with the second principle of Fick's diffusion, which states that the ideal equilibrium between the plant sample and the extraction solvent will be attained at a specific point in the extraction process. Thus, the yields typically drop after reaching ideal equilibrium [26]. This outcome is congruent with the findings of a study that extracted tanshinones from *Salvia miltiorrhiza bunge* using Soxhlet extraction and discovered that the recovery of extraction of all tanshinones increased from 30 to 90 min and decreased above 90 min [36]. In conclusion, the Soxhlet extraction time has a substantial impact on the extraction of the phenolic compounds and the recovery yield from the leaves of *C. gileadensis*. Therefore, 90 min was selected as the best extraction time for the next OFAT to assess the influence of the ratio of the sample to solvent.

Table 1. Impacts of Soxhlet extraction parameters on the TFC, TPC, and extract recovery from *C. gileadensis* leaf.

Soxhlet Extraction Parameters			
Extraction time (min)	$Yield_{Extract}$ (% w/w)	TPC (mg GAE/g d.w)	TFC (mg CE/g d.w)
30	17.30 ± 0.09	39.12 ± 0.89	12.83 ± 0.98
60	18.60 ± 0.26	44.09 ± 1.14	14.45 ± 1.42
90	* 19.50 ± 0.15	* 48.22 ± 1.05	* 15.81 ± 1.19
120	17.50 ± 0.20	41.92 ± 2.02	13.74 ± 2.01
150	16.90 ± 0.21	34.82 ± 1.24	11.42 ± 1.61
Feed/solvent ratio effect (g/mL)	$Yield_{Extract}$ (% w/w)	TPC (mg GAE/g d.w)	TFC (mg CE/g d.w)
1:20	19.10 ± 0.15	43.02 ± 1.09	13.43 ± 0.89
1:25	19.70 ± 0.11	47.53 ± 1.43	15.58 ± 1.64
1:30	* 20.80 ± 0.18	* 52.68 ± 2.01	* 17.27 ± 1.18
1:35	18.30 ± 0.12	49.44 ± 1.16	16.21 ± 2.10
1:40	17.20 ± 0.20	44.44 ± 1.28	14.57 ± 1.39
Ethanol Concentration (% v/v)	$Yield_{Extract}$ (% w/w)	TPC (mg GAE/g d.w)	TFC (mg CE/g d.w)
20	20.60 ± 0.08	49.08 ± 1.69	16.09 ± 1.24
40	* 23.20 ± 0.10	* 59.93 ± 1.33	* 19.65 ± 1.77
60	21.70 ± 0.21	52.87 ± 2.32	17.33 ± 2.15
80	20.90 ± 0.13	47.28 ± 1.51	15.50 ± 1.13
100	19.80 ± 0.09	37.03 ± 2.05	12.14 ± 1.56

* Results are expressed as means ± standard deviation, and $p < 0.05$.

3.2. Sample/Solvent Effect

Another significant parameter is the extraction solvent volume; a higher extraction solvent volume necessitates a longer condensing time and more energy during the purifying process. For greater recovery during the extraction process, the plant matrix must be completely submerged in the solvent. In general, using a larger amount of solvent in traditional extraction procedures might result in better extraction yields [33]. The impact of the feed/solvent ratio on the extract of the *C. gileadensis* leaves, in terms of recovery yields and phenolic compounds, was investigated. The feed/solvent ratio was adjusted to 1:20, 1:25, 1:30, 1:35, and 1:40 g/mL, with a constant extraction time of 90 min and 20% v/v ethanol concentration. Table 1 shows that when the feed/solvent ratio rises to 1:30 g/mL from 1:20 g/mL, the extraction yield and phenolic constituents tend to rise. Then, the yields decreased as the feed/solvent ratio increased above 1:30 g/mL ratio. Thus, at the sample/solvent ratio of 1:30 g/mL, the highest recovery TFC, TPC, and extraction yields from the leaves of *C. gileadensis* were obtained (Table 1). Likewise, the highest recovery yields were achieved with an optimal feed/solvent ratio of 1:30 g/mL, yielding 20.80 ± 0.18% w/w for the extraction yield, 52.68 ± 2.01 mg GAE/g d.w. for the TPC, and 17.27 ± 1.18 mg QE/g d.w. for the TFC. This ratio was statistically more successful than other assessed ratios, as confirmed by one-way ANOVA and validated using a student t-test ($p < 0.05$). These results demonstrate that a 1:30 g/mL feed/solvent ratio is ideal for maximizing phytoconstituent yields. Hence, the 1:30 g/mL ratio of the feed to solvent was selected for the next parameter (ethanol concentration).

3.3. Ethanol Concentration Effect

Ethanol, which is commonly utilized to extract phenolic chemicals from plant matrices, has been discovered to be a non-toxic green solvent. Ethanol is a low-polar solvent that can be mixed in any amount of water [37,38] Therefore, for the extraction of TFC, TPC, and extract yields from the leaves of *C. gileadensis*, the concentrations of ethanol were adjusted at various values from 20 to 100% v/v, whereas the 1:30 g/mL feed/solvent ratio and 90 min of extraction time were kept constant. It is obvious that the TFC, TPC, and yields of extracts gradually rose as the ethanol concentration increased to 40 from 20% v/v; however, the increment over this level of the concentration of ethanol led to remarkable decline in the yield, TPC, and TFC (Table 1). This might be explained by how soluble phenolic chemicals are, which relies on the chemical composition of the sample of plants and the solvent's polarity [37]. As a result, it has been recommended that the use of binary solvent is preferable to mono-solvent in the phenolic constituents' extraction from the plant matrix. Hydrophilic antioxidants are more easily leached when water is present during the extraction process. Additionally, an ethanol concentration of 40% v/v was found to be optimal, yielding 23.20 ± 0.10% w/w for the extract yield, 59.93 ± 1.33 mg GAE/g d.w. for the TPC, and 19.65 ± 1.77 mg QE/g d.w. for the TFC. One-way ANOVA and a student t-test ($p < 0.05$) confirmed the statistical significance and reliability of this finding, establishing 40% v/v as the optimal ethanol concentration for extracting the highest yields of phytoconstituents and lending strong support to our investigation's findings. Thus, the best TPC, TFC, and extraction yield from the extract of leaves of *C. gileadensis* were therefore obtained at the 40% v/v ethanol concentration. These yields were 23.20 ± 0.10% w/w, 59.93 ± 1.33 mg GAE/g d.w., and 19.65 ± 1.77 mg QE/g d.w., respectively.

Overall, the results of this study showed that the extraction of *C. gileadensis* leaf using the Soxhlet extraction method achieved better extraction yields after 90 min of extraction using a feed/solvent ratio of 1:30 g/mL and a 40% v/v ethanol concentration. We observed an extraction yield of 23.20 ± 0.10% w/w, TPC of 59.93 ± 1.33 mg GAE/g d.w., and TFC of 19.65 ± 1.77 mg QE/g d.w. These results were significantly higher than the results obtained via other conventional methods, such as the solvent extraction method using methanol by [20], where the TPC yield was only 20.97 mg GAE/g d.w and the TFC only 6.90 mg QE/g d.w, from the same plant leaf. This reflects the advantage of using the Soxhlet

extraction process over the conventional methods, such as the solvent extraction method, to achieve better recovery yields from the leaf of *C. gileadensis*.

4. Characterization of Extracts

The *C. gileadensis* leaf extract from the Soxhlet extraction under the best OFAT conditions (40% *v*/*v* ethanol concentration, 1:30 g/mL F:S ratio, and 90 min of extraction time) was characterized further as follows:

4.1. FTIR Analysis

One of the well-known methods for identifying and clarifying functional groups in plant extracts is Fourier transform infrared. This characterization was employed for identifying the functional groups in the leaf extract of *C. gileadensis* at maximal conditions according to the peak value in the infrared radiation area. Figure 1 demonstrates the characteristic absorption peaks. Phenolic chemicals were present, as shown by the broad peak at 3317.17cm^{-1}. The bands at 2980.65 cm^{-1}, 2910.45 cm^{-1}, and 2123.72 cm^{-1} reflect the primarily *vas*(CH_2) and *vs*(CH_2) stretching, which show the existence of lipid–carbohydrate molecules, whereas the bands at 1637.50 cm^{-1}, 1445.87 cm^{-1}, 1386.54 cm^{-1}, and 1334.89 cm^{-1} demonstrate the existence of phosphodiester stretching, which proves that nucleic acid exists, as well as *vas*(>P=O), C=O stretching, N–H bending, and carboxylic acid bending [39]. At 1266.04 cm^{-1}, the band indicates an abundance of C–O groups in polyols, which include hydroxy-flavonoids [40]. The peaks at 1084.98 cm^{-1} and 1043.88 cm^{-1} could be related to the secondary alcohols or the ester group. Additionally, the vibration of aromatic rings may be the cause of the peak at 876.83 cm^{-1}. Therefore, the unique fingerprints discovered in the extract prepared from the *C. gileadensis* leaves using the Soxhlet method suggested the existence of functional features connected to flavonoids and polyphenols.

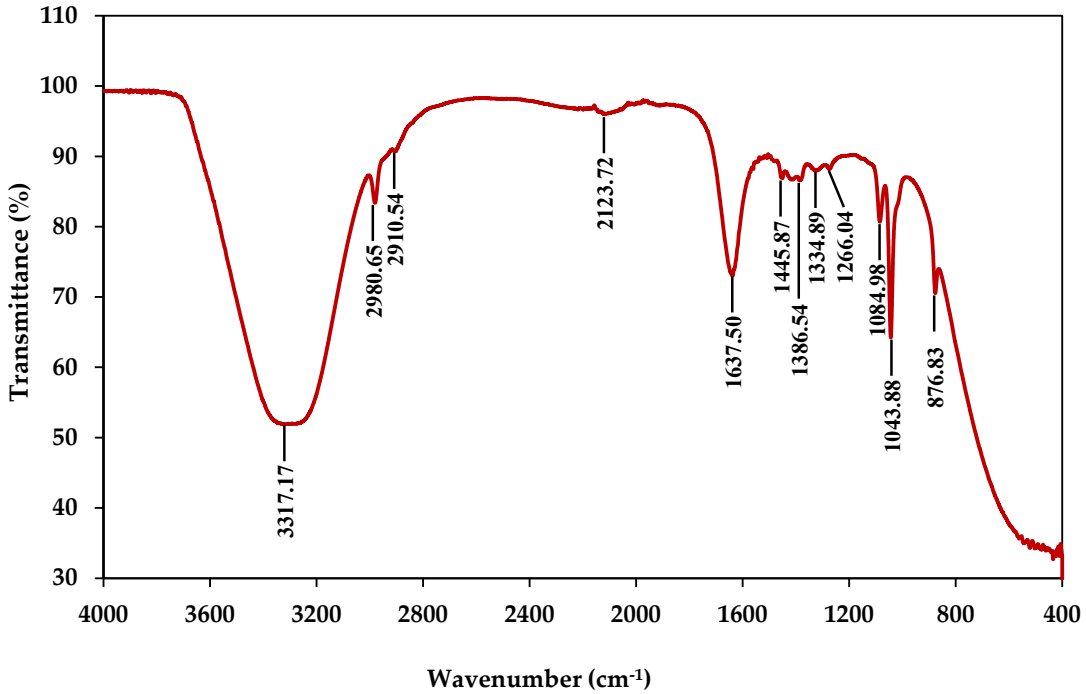

Figure 1. IR spectra of *C. gileadensis* leaf extract from Soxhlet extraction method.

4.2. GC–MS Analysis

Recent developments in the manner of life have altered the nutritional value of the food consumed and raised worries about how industrially manufactured goods can impact the health of people. This is due to the fact that the majority of consumer goods are made using synthetic materials, many of which are known to be harmful to human health. Instead of manufactured alternatives, raw materials from nature are sought as a replacement. In

this sense, extracts encounter greater demand than synthetic additives because they are frequently used in food or traditional medicine. The chemical components of leaf extract of *C. gileadensis* extracted using the Soxhlet technique have been determined by GC–MS analysis, as shown in Figure 2 and Table 2.

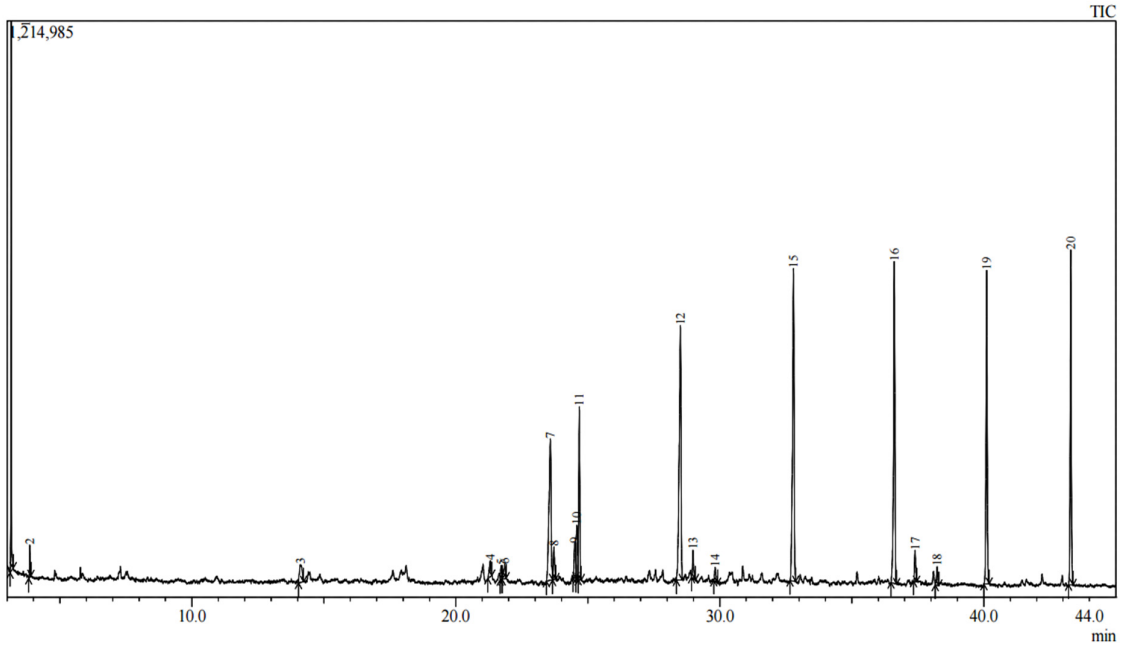

Figure 2. GC–MS spectra of *C. gileadensis* leaf extract from Soxhlet extraction method.

Table 2. Tentatively identified phytochemicals of leaf extract of *C. gileadensis* via GC–MS analysis.

Peak	Compound Name	Peak Area (%)	Mol. Formula	Mol. Weight	R.T (min)
1.	2,3-Pyridinedicarboxylic anhydride	7.43	$C_7H_3NO_3$	149	3.156
2.	Ethanol, 2-(trimethylsilyl)-	0.56	$C_5H_{14}OSi$	118	3.856
3.	1-Dodecene	1.44	$C_{12}H_{24}$	168	14.107
4.	Undecane, 2-methyl-	0.75	$C_{12}H_{26}$	170	21.303
5.	Naphthalene, 1,7-dimethyl-	0.60	$C_{12}H_{12}$	156	21.721
6.	Naphthalene, 2,6-dimethyl-	0.79	$C_{12}H_{12}$	156	21.846
7.	Cycloheptasiloxane, tetradecamethyl-	8.84	$C_{14}H_{42}O_7Si_7$	518	23.580
8.	Undecay alcohol	1.21	$C_{11}H_{24}O$	172	23.716
9.	Silane, trichlorooctadecyl-	1.69	$C_{18}H_{37}C_{13}Si$	386	24.495
10.	Phenol, 2,4-bis(1,1-dimethylethyl)-	2.47	$C_{14}H_{22}O$	206	24.582
11.	Pentanoic acid	5.42	$C_{19}H_{30}O_3$	306	24.676
12.	Cyclooctasiloxane, hexadecamethyl-	13.87	$C_{16}H_{48}O_8Si_8$	592	28.506
13.	2-Naphthalenemethanol, decahydro-.alpha.,alpha.,4a-trimethylene-	1.02	$C_{15}H_{26}O$	222	28.984
14.	1-Tetradecanol	0.57	$C_{14}H_{30}O$	214	29.823
15.	Cyclononasiloxane, octadecamethyl-	14.78	$C_{18}H_{54}O_9Si_9$	666	32.791
16.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexxadecamethyl-	13.70	$C_{16}H_{50}O_7Si_8$	578	36.607
17.	1-(+)-Ascrobic acid 2,6-dihexadecanoate	1.08	$C_{38}H_{68}O_8$	652	37.398
18.	Docosanoic acid, ethyl ester	0.68	$C_{24}H_{48}O_2$	368	38.231
19.	Heptasiloxane, hexadecamethyl-	11.47	$C_{16}H_{48}O_6Si_7$	532	40.107
20.	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	11.62	$C_{13}H_{40}O_5Si_6$	444	43.293

Table 2 shows the identification of 20 phytoconstituents in the extract of the *C. gileadensis* leaves; these compounds belong to the hydrocarbons, alcohols, esters, and fatty acids groups. Among these compounds, the most abundant are 2,3-Pyridinedicarboxylic anhydride (7.43%), which has antibacterial, antiepileptic, and antitumor activities [41]; Cycloheptasiloxane, tetradecamethyl (8.84%), which reportedly possesses antimicrobial and anticancer activity [42]; and Heptasiloxane, hexadecamethyl (11.47%), reported to have antifungal, anti-inflammatory, antiarthritic, antimicrobial, antioxidant, antiasthma, diuretic, and analgesic properties [43]. Also, 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane (11.62%) possesses antimicrobial activity [44]; Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (13.70%) demonstrated antibacterial and antifungal effects [45,46]; Cyclooctasiloxane, hexadecamethyl (13.87%) has antimicrobial, and antioxidant activities [47,48]; and Cyclononasiloxane, octadecamethyl (14.78%) has anti-fungal activities [49]. It is notable that these phytochemicals from *C. gileadensis* leaf extracts obtained through Soxhlet extraction contained a diverse set of bioactive phytoconstituents with potent antioxidant activity.

5. Conclusions

This study examined the impact of the factors of Soxhlet extraction on the TFC, TPC, and recovery yield from *C. gileadensis* leaves using the OFAT method. The best TFC, TPC, and extraction yield were attained at the following process conditions: process = Soxhlet extraction, process time = 90 min, sample/solvent ratio = 1:30 g/mL, and solvent concentration = 40% v/v. The obtained values were as follows: yield = 23.20 $\pm$ 0.10% w/w, TPC = 59.93 $\pm$ 1.33 mg GAE/g d.w., and TFC = 19.65 $\pm$ 1.77 mg QE/g d.w. Furthermore, the GC–MS analysis of the extract discovered the presence of 20 phytoconstituents, with the following being the most abundant: Cyclononasiloxane, octadecamethyl (14.78%); Cyclooctasiloxane, hexadecamethyl (13.87%); Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (13.70%); Heptasiloxane, hexadecamethyl (11.47%); 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane (11.62%); Cycloheptasiloxane, tetradecamethyl (8.84%); and 2,3-Pyridinedicarboxylic anhydride (7.43%). Moreover, the extract from *C. gileadensis* leaves displays significant antioxidant activity which could be potentially employed as a natural antioxidant. Given this, our discoveries may have important industrial applications in the pharmaceutical field. Furthermore, subsequent research endeavors may involve increasing the extraction process's scale and assessing the phytoconstituents' effectiveness in pharmaceutical formulations.

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