ISSN (Print) 2794-7629
ISSN (Online) 2794- 4549
Received 12/01/2025
Accepted 24/01/2026

FULL PAPER

Antioxidant Evaluation and Spectroscopic Characterisationof *Commiphora gileadensis* Extract: Implications of Thermal Maceration on Phenolic Recovery

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Running title:
Antioxidant activity of *C. gileadensis*

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Abstract

Commiphora gileadensis (L.) C.Chr, indigenous to the Middle East, is a tree with a long history in traditional healing practices. This investigation aims to evaluate the phytochemical profile and antioxidant potential of a 50% ethanolic extract derived from the bark of this species. The extract was prepared via maceration and subsequently analysed using FTIR and UV-Vis spectroscopy to identify functional groups associated with bioactive constituents. Quantitative assessments of total phenols and flavonoids were conducted using standard colourimetric assays, while the antioxidant capacity was determined through the DPPH radical scavenging method. The extract demonstrated strong radical-scavenging abilities, achieving an EC_{50} of 13.22 ± 0.46 µg/mL. The Total Phenolic Content (TPC) was 12.56 ± 0.19 mg GAE/g, a result influenced by the thermal extraction process. Spectroscopic data confirmed the presence of functional groups typical of phenolic and flavonoid compounds. Although thermal maceration produced an extract with potent antioxidant activity, the phenolic yield was lower than that of modern non-thermal methods. These findings suggest that while the bark is a valuable source of stable antioxidants, yield could be improved through optimised extraction techniques.

Keywords: *Commiphora gileadensis* bark; ethanolic extract; DPPH antioxidant activity; phytochemicals analysis

Introduction

The search for new drugs in natural products has its roots in the long history of using plants for medicine.1 *Commiphora gileadensis* (*C. gileadensis*) is a member of the Burseraceae family. It is native to the Middle East, especially Saudi Arabia and Yemen, where it plays an important role in traditional medicine [2–4]. People often use the bark to treat burns, wounds, and scorpion stings in traditional ways. Also, it is known to have a wide range of pharmacological effects, such as being an analgesic, anti-inflammatory, and antimicrobial [5–7].

Researchers have already shown that the methanolic extract of bark can kill bacteria that are resistant to drugs, such as MRSA and Pseudomonas aeruginosa.[2] In the same way, extracts from the leaves have been shown to have strong antioxidant properties, significant DPPH inhibition, and cytotoxic effects against HepG2 liver cancer cells.3 We know a lot about the resin, but we need to learn more about the bark's phytochemical complexity, especially when it comes to its lipid and phenolic components like ceramides and tannins.[2] Traditional extraction methods, like maceration, are still popular because they are easy to use and don't cost much. They usually use organic solvents to separate these bioactive compounds.[4] The Burseraceae family is known for having a lot of polyphenols, which is what makes them good at fighting bacteria and acting as antioxidants.[6] The presence of aromatic hydroxyl groups and conjugated double bonds, which help with radical scavenging, is often linked to how well these agents work.[8] Finding new sources of antioxidants is very important for improving human health outcomes because oxidative stress plays a role in the development of chronic diseases like cancer and aging.[10] As a result, plant extracts that are high in phenolics and flavonoids are being looked at more closely for their possible uses in the pharmaceutical and nutraceutical industries [16–18].

Recent studies have shown that the phytochemicals in *C. gileadensis* are very sensitive to different ways of extracting them. For example, Bin Mokaizh et al. (2024) showed that advanced methods like Ultrasonic-Assisted Extraction (UAE) can greatly improve the recovery of phenolics from the bark compared to more traditional methods. Also, Shadid et al. (2023) used LC-MS/MS profiling to find certain bioactive compounds in the aerial parts, such as hesperidin and quercetin derivatives, that add to the plant's pharmacological profile. So, it is very important to know how traditional thermal processing affects these bioactive compounds in order to prove that ethnobotanical uses are correct.

The main goal of this study was to use the DPPH test to find out how well the 50% ethanolic extract of *C. gileadensis* bark could get rid of free radicals. The study also wanted to use spectroscopic methods (FTIR and UV-Vis) to describe the phytochemical makeup and measure the total amount of phenolic and flavonoid compounds.

Methods

Materials and instrumentation:

Spectroscopic measurements were performed using a Thermo Nicolet Nexus 670 FTIR spectrophotometer (Thermo Scientific, USA) equipped with OMNIC software, and a Lambda 25 UV/Vis spectrophotometer (PerkinElmer, USA) utilising UV WinLab V2.85 software. Extraction and processing utilised a rotary evaporator (Eyela, USA) and a freeze dryer (Labconco, USA). Absorbance for assays was measured using a microplate reader (Thermolab Systems, Finland). All chemicals, including DPPH, gallic acid, quercetin, Folin-Ciocalteu reagent, and solvents, were analytical grade and sourced from Sigma-Aldrich, USA.

Plant collection and authentication:

Bark samples of *C. gileadensis* were harvested from the Madodah region in Seiyun, Hadhramout, Yemen (Altitude: 600 m; 15.56° N, 48.56° E).11 Taxonomic identification was confirmed by the Agricultural Research Station in Seiyun and validated by the Herbarium Department at Universiti Sains Malaysia (Voucher No. 11558-*Commiphora gileadensis*-2015). Prior to extraction, the bark was cleaned, rinsed with distilled water, dried at 37ºC for 48 hours, and ground into a powder.

Preparation of 50% ethanol extract:

The extraction process utilised a 50% ethanol solvent via maceration. Initially, 100 g of dried, pulverised *C. gileadensis* bark was submerged in 500 mL of a 1:1 ethanol-water mixture. This mixture was maintained at 60°C with constant agitation (100 rpm) for a duration of 48 hours. This thermal maceration protocol (60°C for 48 hours) was selected to simulate the harsh conditions often found in traditional herbal preparations, thereby allowing for the evaluation of the antioxidant capacity of the thermally stable phytochemical fraction.[4]

Following extraction, the mixture was filtered, and the filtrate was concentrated using a rotary evaporator at 70°C. The resulting concentrate was freeze-dried at -50°C to yield a dry powder. The final yield was calculated as a percentage of the initial dry weight. The dried extract was stored in airtight containers for subsequent analysis.

Stock Solutions:

For analysis, stock solutions (10 mg/mL) of the extract and standards (gallic acid, quercetin) were prepared in methanol. Reagents for the colourimetric assays were prepared in distilled water.

Fourier transform infrared spectrophotometry (FTIR):

Functional group analysis was conducted using the KBr pellet technique. The FTIR spectrum was recorded in the range of 4000–400 cm^{-1} at room temperature to identify characteristic vibrational bands of the phytoconstituents.[12]

Ultraviolet visible (UV-Vis) spectrophotometry:

To assess the conjugation systems, the extract (25 µg/mL in methanol) was scanned from 200 to 500 nm using a quartz cuvette (1 cm path length). The spectrum was analysed for maximum absorption peaks (λmax) indicative of specific chromophores.[14]

DPPH Radical Scavenging Assay:

The antioxidant potential was measured by the extract's ability to bleach the purple DPPH solution, following established protocols.16 A 100 µL aliquot of the extract at various concentrations (ranging from 2.5 to 80 µg/mL) was mixed with an equal volume of 100 µM DPPH in methanol within a 96-well plate. Gallic acid was employed as the positive standard. After incubating the mixture at 37°C for 35 minutes, absorbance was read at 517 nm against a methanol blank. The scavenging activity was calculated as a percentage of inhibition, and the EC_{50} value (concentration required to inhibit 50% of radicals) was derived from regression analysis of the dose-response curves.

Total Phenolic Content (TPC):

Phenolic quantification was performed using the Folin-Ciocalteu method.13 Briefly, 100 µL of the extract (1 mg/mL) was mixed with 750 µL of diluted Folin-Ciocalteu reagent (1:10 ratio). After standing for 5 minutes in the dark, 750 µL of sodium bicarbonate (60 g/L) was added. The reaction mixture was incubated at 30°C for 30 minutes, after which absorbance was recorded at 725 nm. TPC was derived from a gallic acid calibration curve and reported as mg Gallic Acid Equivalents (GAE) per gram of sample.

Total Flavonoid Content (TFC):

Flavonoid levels were assessed using the aluminium chloride colourimetric assay.13 A 0.5 mL sample of the extract was mixed with 1.5 mL of methanol, followed by the addition of 0.1 mL of aluminium chloride (10%), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was allowed to react at room temperature for 30 minutes. Absorbance was subsequently measured at 415 nm. Quercetin served as the standard, and results were expressed as mg Quercetin Equivalents (QE) per gram.

Statistical analysis:

Data are presented as mean ± standard deviation (SD) of triplicate measurements. Statistical significance was evaluated using one-way ANOVA followed by Dunnett's post-hoc test (SPSS v20), with $p < 0.05$ considered significant. Regression analysis was used to calculate EC_{50} values.

Results

The extraction process yielded 8.23% (w/w) of dry extract. The sample exhibited potent radical-scavenging activity, along with appreciable levels of phenolics and flavonoids.

FTIR analysis:

The FTIR spectrum (Table 1, Figure 1) revealed characteristic absorption bands. notably a broad band at 3391 cm^{-1} corresponding to O-H stretching, and signals at 2921 cm^{-1} (C-H stretching) and 1614 cm^{-1} (C=C aromatic stretching), confirming the presence of phenolic and aromatic structures.[18]

Table 1 Analysis of FTIR spectrum of most transmittance bands in correlation to the corresponding wavenumbers, which represent the functional organic groups in the 50% ethanol extract [27].

Vibrational frequency (cm^{-1})	Corresponding organic groups
3391 (broad)	O-H stretching
2921	C-H stretching
2353	P-H or $C=NH^{+}$ stretching
1744 (weak)	C=O, C=C stretching
1614 (Strong)	NO_2- stretching
1552 and 1516	C-H bending (deformation)
1287, 1111, and 1046	Mixed, C-C, C-O, C-N stretching
927	C-H bending
551 (Broad)	C-X stretching (Aliphatic, X: Cl, Br, or I)

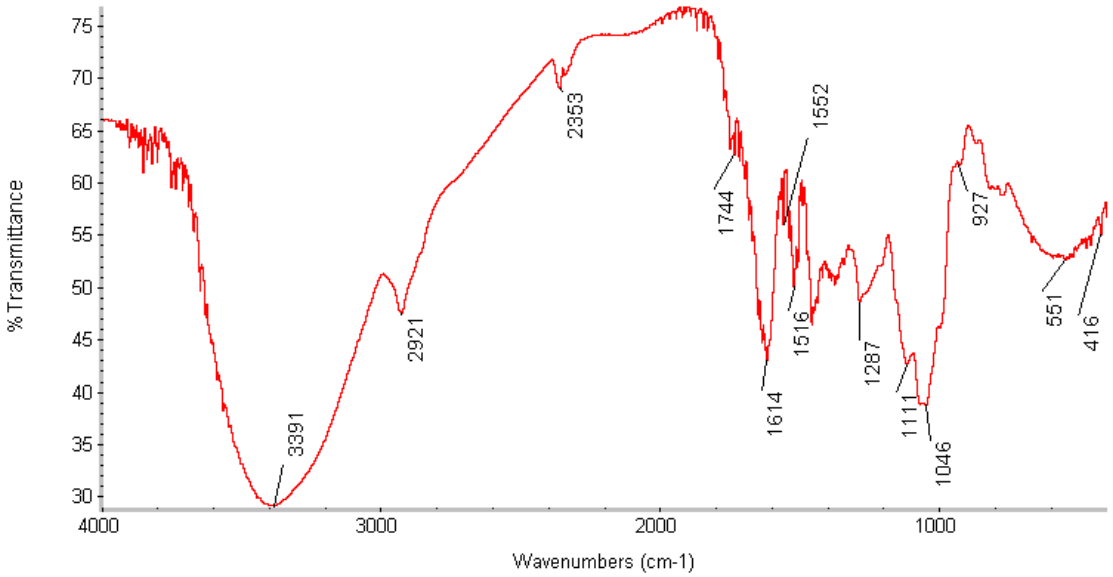


Figure 1 FTIR fingerprint of the extract.

UV-Vis spectrophotometry analysis:

The UV-Vis spectrum (Figure 2) showed absorption maxima at 204 nm and 281 nm. The peak at 281 nm is characteristic of the benzoyl system in flavonoids and phenolic acids, indicating the presence of conjugated π-systems.[14]

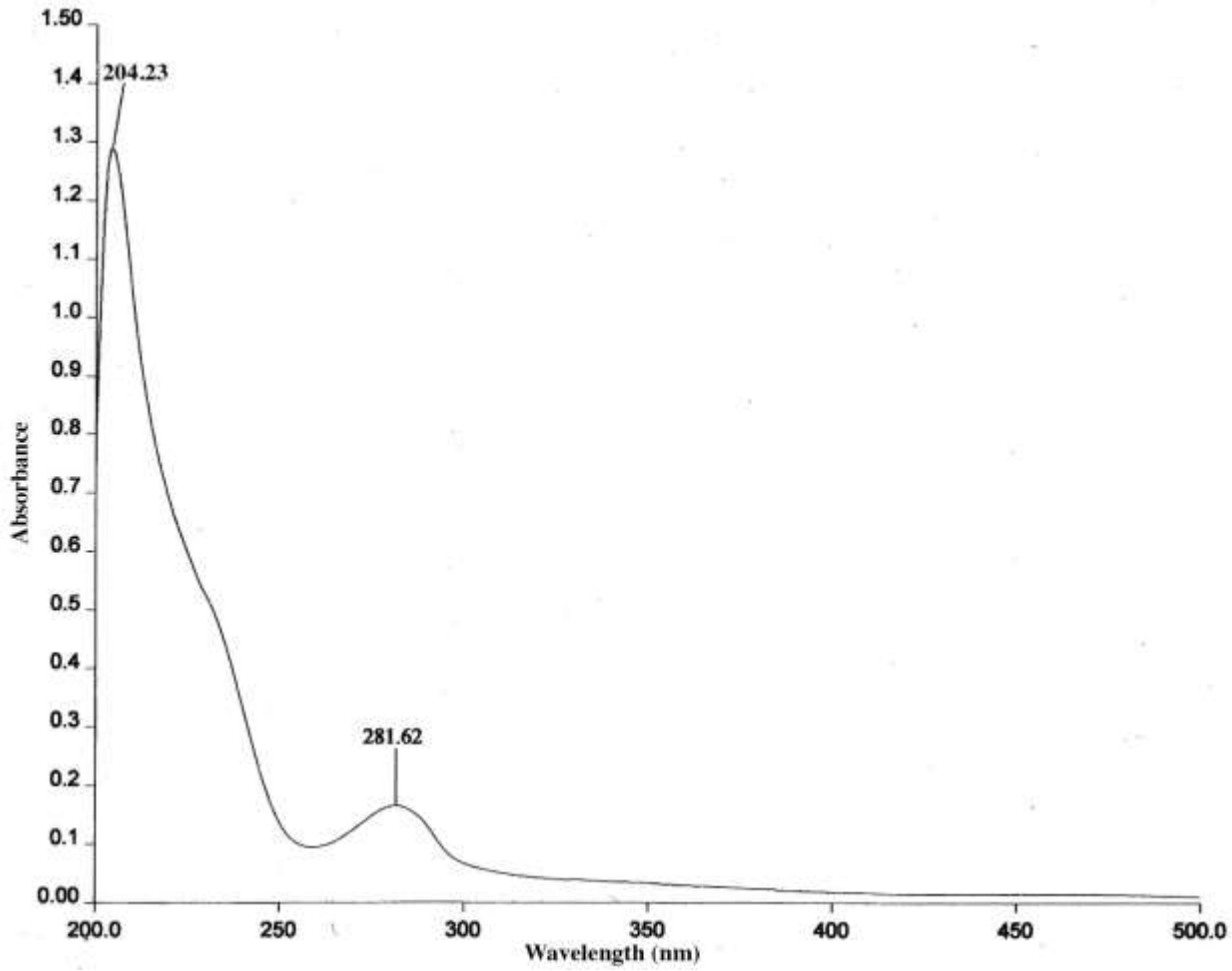


Figure 2 UV-Vis absorption spectrum of the extract in the range of 200-500 nm.

DPPH free radical antioxidant scavenging activity:

The extract displayed dose-dependent antioxidant activity (Table 2). At 80 µg/mL, the inhibition was 94.05%, comparable to the standard. The calculated EC_{50} for the extract was 13.22 ± 0.46 µg/mL, indicating potent activity compared to gallic acid (EC_{50} 0.619 ± 0.051 µg/mL).

Table 2 Percentages of DPPH free radical scavenging activity (antioxidant activity) of the extract at various concentrations (2.5, 5, 10, 20, 40, 60, and 80 µg/mL) as mean ± SD of three experiments.

Concentrations (µg/mL)	DPPH free radical scavenging activity (%)
2.5	7.21±2.35
5	15.06±1.38
10	33.12±2.09
20	65.06±1.38
40	92.62±0.33
60	92.84±1.01
80	94.05±0.39

$P < 0.05$ in comparison to the negative control (methanol).

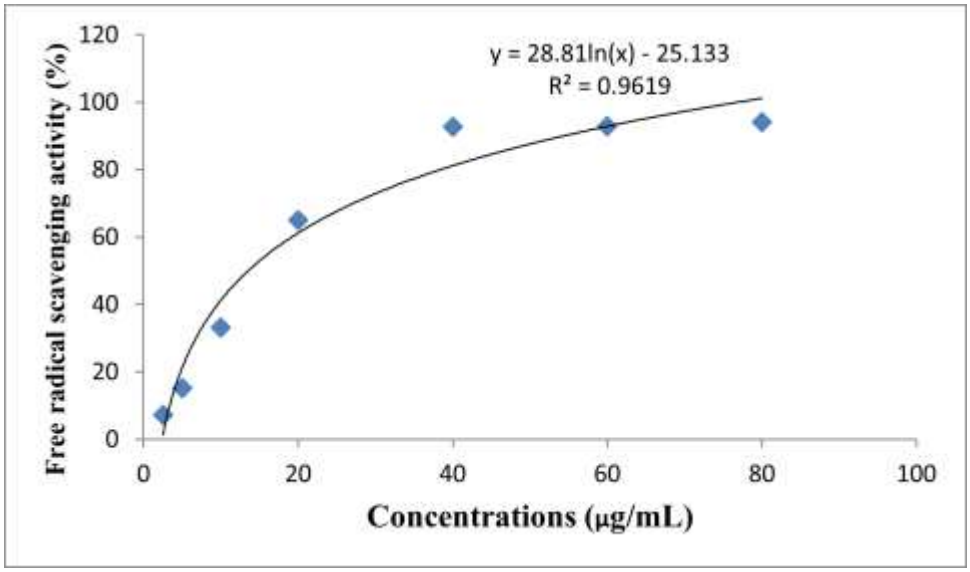


Figure 3 Concentration-activity dependence of various concentrations of the extract (2.5, 5, 10, 20, 40, 60, and 80 µg/mL). Results are expressed as the percentage of DPPH free radical scavenging activity against the concentration of the extract (n=3). $P < 0.05$ in comparison to the negative control (methanol).

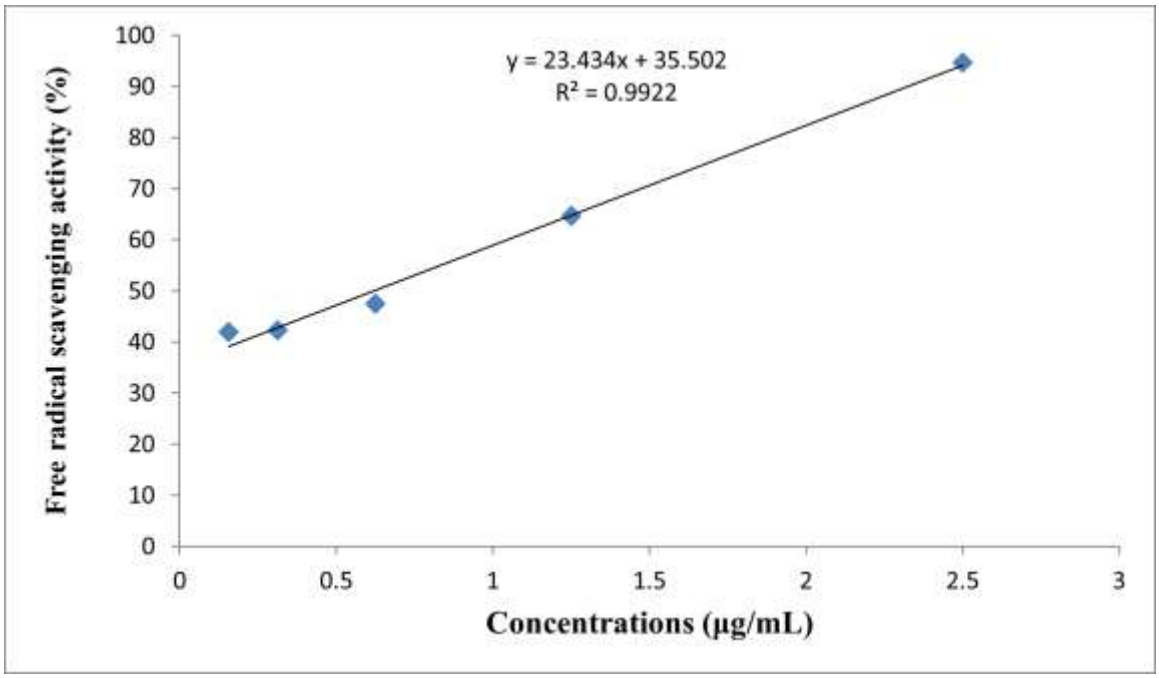


Figure 4 Concentration-activity dependence of various concentrations of gallic acid (0.156, 0.313, 0.625, 1.25, 2.5, 5, and 10 µg/mL). Results are expressed as the percentage of DPPH free radical scavenging activity against the concentration of gallic acid (n=3). *P* < 0.05 in comparison to the negative control (methanol).

Total Phenolic and Flavonoid Content:

The TPC was determined to be 12.56 ± 1.20 mg GAE/g, while the TFC was found to be 13.17 ± 0.75 mg QE/g. These values indicate a moderate recovery of bioactive compounds under the tested conditions.

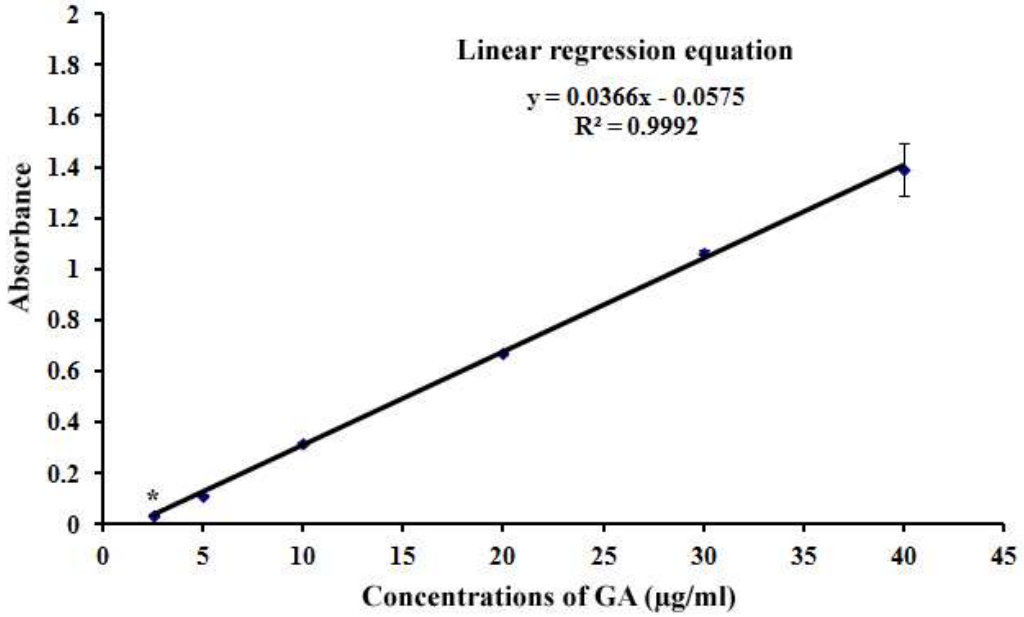


Figure 5 Standard calibration curve and linear regression equation of gallic acid for the total phenolic content study, *$P > 0.05$.

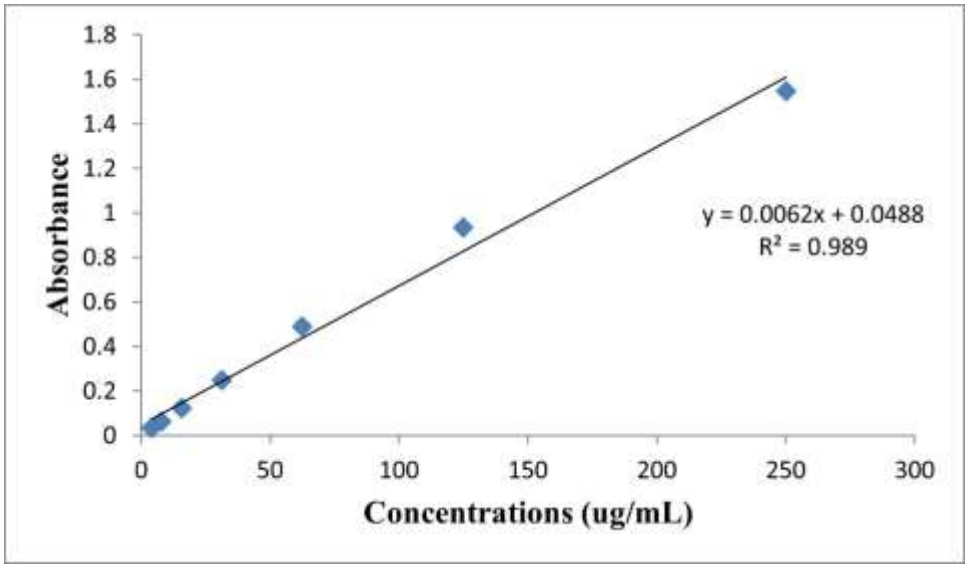


Figure 6 Standard calibration curve and linear regression equation of quercetin for the total flavonoid content study.

Discussion

Natural products are still an important part of traditional medicine for treating symptoms and managing chronic illnesses.[15] The goal of this study was to confirm that *C. gileadensis* is bioactive. In ethnopharmacology, it is known for its ability to heal wounds and ease pain [2]. Spectroscopic profiling and antioxidant tests were used to do this. The extraction yield of 8.23% shows that the polar solvent system (50% ethanol) and heat processing worked well together to break down a lot of the bark's matrix. This backs up the idea that raising the temperature and stirring for longer periods of time speeds up mass transfer, but it might also make some chemicals that break down at high temperatures less stable.

The Total Phenolic Content (TPC) measured in this study (12.56 ± 0.19 mg GAE/g) is different from what has been published recently. For instance, Bin Mokaizh et al. (2024) found that the TPC values for *C. gileadensis* bark obtained using Ultrasonic-Assisted Extraction were much higher, at about 149.75 mg GAE/g. The difference suggests that certain thermolabile polyphenols may have broken down after being exposed to 60°C for a long time during maceration. This is something that has been well-documented in flavonoid extraction.[4] The extract, on the other hand, still had a lot of antioxidant activity (EC50 13.22 µg/mL). This suggests that the bioactivity that stays is probably due to thermally stable flavonoid glycosides or the creation of Maillard reaction products that are active as antioxidants after heating, not just the amount of phenolics present.

Flavonoids are important for keeping cells safe because they stop free radicals and lipid peroxidation.[21] It is very important to lower oxidative stress in order to avoid diseases like cancer.[23] Its activity is very high (EC_{50} 13.22 µg/mL), which is interesting because leaf extracts usually need bigger doses to work.[3] This implies that the bark may accumulate certain antioxidant reserves. The FTIR test of hydroxyl and carbonyl functionalities strengthens the chemical basis for this activity.

Conclusion:

The 50% ethanolic extract of *C. gileadensis* bark exhibits potent antioxidant activity (EC50 13.22 µg/mL) despite the moderate phenolic yield obtained via thermal maceration. While the extraction method likely limited the recovery of total phenolics compared to modern ultrasonic techniques, the results confirm that the bark contains a robust fraction of thermally stable antioxidants. Future research should utilise LC-MS/MS profiling to identify these specific stable compounds and explore non-thermal extraction methods to maximise phytochemical yield.

Conflict of Interests:

All authors in this study declare no potential conflicts of interest.

Acknowledgements:

The authors express their sincere gratitude to the School of Pharmaceutical Sciences, Universiti Sains Malaysia, for providing laboratory facilities and technical support throughout this study. Special appreciation is extended to the Agricultural Research Station in Seiyun, Yemen, and the Herbarium Department at the School of Biological Sciences, Universiti Sains Malaysia, for their cooperation in the taxonomical authentication of the plant.

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