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Research Article

Isolation and identification of phenolic compounds in guava leaves and assessment of their cytotoxic effects against AMJ-13 and MCF-7 breast cancer cell lines

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Abstract:

Background: *Psidium guajava*, commonly known as guava, is a tropical tree prized for its nutritious fruit and medicinal properties. This member of the Myrtaceae family is rich in phytochemicals, which are natural compounds with potential health benefits. Studies have shown that guava leaves and fruits possess various pharmacological activities, including anti-cancer properties against several cancer cell lines.

Objectives: This study aimed to isolate and identify phenolic compounds in guava leaves and assess their cytotoxic activity against AMJ-13 and MCF-7 cell lines by ethyl acetate fraction of guava leaves grown in Iraq.

Methods: The researchers will employ High-performance liquid chromatography and Fourier transform infrared techniques to analyze the guava leaf extract. Subsequently, preparative High-performance liquid chromatography was used to isolate the specific phenolic compounds of interest, and a colorimetric MTT reduction assay, was conducted using guava extract to assess their effects on human cancer cell lines. Analysis using GraphPad Prism software version 6.

Results: The researchers successfully isolated pure samples of caffeic acid, luteolin, and Gallic acid, which are all flavonoids, from the guava leaf extract using preparative High-performance liquid chromatography. These isolated phenolic compounds were from ethyl acetate fraction (F3). This enriched fraction was tested for its cytotoxic activity against the Iraqi AMJ-13 and human MCF7 breast cancer cell lines. The results showed a decrease in cell viability, indicating the fraction's potential anti-cancer properties. The fraction was more effective against the Iraqi AMJ-13 cells with an IC50 value of 414.3 µg /ml, compared to the human MCF7 cells with an IC50 value of 698.3 µg /ml.

Conclusion: The analytical techniques used in this study, like HPLC and FTIR, provided a detailed profile of the phenolic compounds present in guava leaves. This information, combined with the cytotoxic tests, suggests that guava leaves have the potential to kill cancer cells in a concentration-dependent manner.

Keywords: Caffeic acid; Gallic acid; Guava; Luteolin; Ultrasonic-assisted extraction.

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Introduction:

Worldwide, the use of traditional medicines has a long history and encompasses an easily accessible and affordable source of treatment (1-3). Many people who live in developed countries depend on traditional medicine (4-6). *Psidium guajava* grows in tropical and subtropical areas of the world and contains several bioactive compounds. It belongs to the family Myrtaceae (7,8). All parts, including the fruits, leaves, and barks have been traditionally used as folkloric herbal medicines and appear in many medicinal uses(9,10). Many previous studies reported many phytochemical constituents in guava leaves that have many pharmacological activities and medicinal properties Various pharmacological reports have verified the capacity of this plant to reveal hepatoprotection, antigenotoxic, anti-bacterial, cytotoxic activity against cancer cell lines, anticough, anti-diabetic and anti-inflammatory events(11-14). Guava has a high content of antioxidant compounds, guava leaves have a higher content of gallic acid, ellagic derivatives quercetin, resveratrol, daidzin, guaijaverin, avicularine, hyperin, morin, chlorogenic acid, rutin, vanillic acid, p-hydroxyl benzoic acid, syringic acid, myricetin, naringenin, and apigenin(15,16). Many previous studies approved the *in vitro* and *in vivo* anti-cancer activities of different fractions and extracts from all guava parts and especially leaves against human breast cancer cell line (17-19). This study focuses on the isolation of phenolic compounds, from *Psidium guajava* (guava) leaves newly cultivated in Iraq and the assessment of the cytotoxic activity of guava leaf ethyl acetate fraction against Iraqi breast cancer cell line and human breast cancer cell line.

Thus, this study was conducted to evaluate the cytotoxic effect of the phenolic compounds of guava leaves on AMJ-13 and MCF-7 cell lines.

Material and method

preparation of plant material **:** In spring 2023, *Psidium guajava* leaves were collected from Musayyib city and first grown in Iraq. After the collection, the plant specimens underwent

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identification and authentication conducted by expert Dr. Sukaena Abass from the College of Sciences. The plant components were thoroughly dried in the shade, finely ground, and stored to use for further extraction and analysis (20). Extraction of guava leaves: Approximately 50 grams of finely ground *Psidium guajava* leaves underwent extraction using an ultrasonic bath sonicator, capitalizing on the cavitation principle to enhance the release of plant compounds. The ultrasonic waves, operating at 40 KHz, dissolved 50 grams of leaf powder in 750 ml of 70% ethanol at 30°C for 40 minutes (21). The crude ethanol extract underwent filtration and concentration under condensed pressure using a rotary evaporator. The dried extract obtained was liquified in 500 ml of distilled water and underwent successive partitioning with 500 ml aliquots of petroleum ether, ethyl acetate, chloroform, and n-butanol. This partitioning process was repeated three times for each solvent in a separatory funnel. All fractions, except n-butanol, underwent drying over anhydrous sodium sulfate, followed by filtration and subsequent evaporation with a rotary evaporator until complete dryness (22).

Preliminary phytochemical assessment: Initial chemical examinations were carried out to decide the incidence or lack of polyphenolic compounds in Iraqi *Psidium guajava* achieved for extracting leaves ethyl acetate fractions (F3) and butanol fraction (F4). On examination done by Alkaline reagent tests, a few amounts of extracts plus drops of sodium hydroxide, and a yellow color appeared. Still, it appeared colorless by adding drops of diluted HCL (23).

Analysis of Guava Fractions by high-performance liquid chromatography and isolation of phenolic compounds by Preparative high-performance liquid chromatography: A qualitative check through HPLC for the (F3) and (F4) fractions of leaves, comparing them to caffeic acid, luteolin, and Gallic acid standards, Sample preparation involved dissolving (F3) and (F4) fractions in methanol. This solution underwent filtration using a filter membrane (0.4 µm) before inoculation into the HPLC column. Matching between samples and standards was achieved based on observed retention times (24). The conditions for the analysis of the (F3) and (F4) fractions by HPLC were conducted using a stationary phase on Knuaer, Germany C18 column (5 µm particle size, 250 x 4.6 mm) with a Dionex Ultimate 3000 liquid chromatograph. The mobile phase consisted of a 1% aqueous acetic acid solution and acetonitrile utilizing gradient elution. The rate of flow was maintained at 0.7 ml/min. The column was thermally regulated at 28°C, and an inoculation volume of 20 µl was employed. The gradient elution proceeded linearly, transitioning from 10% to 40% of the component (25,26). Phytochemical ingredients in plant extracts are often present in small quantities, necessitating a sensitive instrument for recognition and isolation. In this study, Preparative high-performance liquid chromatography was employed for the isolation of phenolic compounds, Preparative high-performance liquid chromatography apparatus specification, and conditions as in previous studies (27,28).

Examination by Fourier transform infrared (FT-IR),

FT-IR spectra of isolated compounds were documented in the FTIR spectrometer (Shimadzu)in the range of wave number 500 to 4000 cm–1 (29).

Cell Line Maintenance

The method of work was conducted for cell line maintenance according to Freshney RI (30).

MTT assay

The F3 fraction cytotoxicity effect on Iraqi breast cancer cell line AMJ-13 and human breast cancer cell line MCF-7 was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay based on the detection of mitochondrial dehydrogenase activity in living cells, first day of poring the cells in a 96-well microplate by counting cells using trypan blue, about 1 x 104 cells were cultured in each well. After the cells were cultivated, the 96-well plate was sited in an incubator at 37 ℃ for 24 hours until 60% of the well surface was filled, otherwise, more time was needed (31).

On the second day, treatment of AMJ-13and MCF-7cell lines with different concentrations (31.2, 62.5, 125, 250, 500, 1000µg/ml), (25, 50, 100, 250, 500, 1000 µg/ml), of guava leaves F3 for 72 h after emptying the supernatant of each well by the sampler, 100 µl of every dilution was added to the wells. The pouring pattern was drawn and eight wells were considered for each dilution. On the third day adding MTT dye 24 hours later, the medium was removed and MTT solution 100 µl (0.5 mg/ml) was added to the plates in the shady and put in the incubator for 4 hours (32). Then, the top medium of the wells was detached with a sampler, and DMSO 100 µl was added to the wells, and placed on a shaker for 20 minutes (at this stage, the container should be hidden so as not to be exposed to light). Finally, the intensity of the resulting color was read by a (DNM-9602G) microplate reader at 570 nm (33).

Statistical analysis

Data were analyzed using GraphPad Prism version 6 and presented as mean ± SD of triplicate measurements. In addition, data were compared using an unpaired *t*-test, and the significance levels were considered at $P < 0.0001$.

Results

Extraction by Ultrasonic bath sonicator

The selected method for leaf extraction was based on ultrasound-assisted extraction (UAE), resulting in a higher percentage yield of approximately 20 %w/w from 50g of guava leaves according to the following formula (34,35): **percentage yield =weight of crude (g)/weight of plant material (g) x100**. This method relies on the principle of cavitation, which enhances the release of compounds from the plant material. The conditions for UAE included ultrasonic waves at 40 KHz, utilizing 50g of leaf powder in 750 ml of 70%

ethanol at 30°C for 40 minutes. Subsequently, the crude ethanol extract underwent filtration and concentration under reduced pressure using a rotary evaporator.

Preliminary phytochemical investigation : Chemical tests were done on the guava leaves and showed the results were positive indicating the presence of phenolic compounds in F3 and F4 fractions.

Analysis of Guava Fractions by High-performance liquid chromatography

Analysis of the F3 and F4 fractions was conducted for leaves using HPLC, as depicted in figure 1.

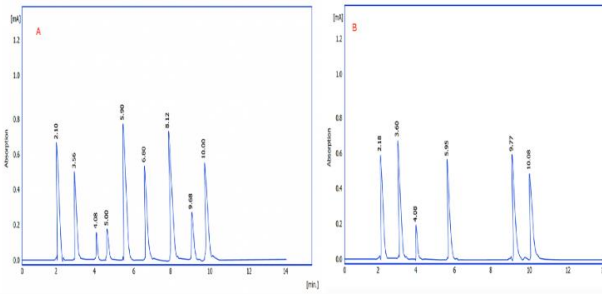


Figure (1): HPLC of guava leaves F3 fraction. HPLC of guava leaves F4 fraction

HPLC examination remarkably close retention time value of caffeic acid standard, luteolin standard, and gallic acid standard matched to the isolated A3, A8, and A2 compounds isolated by PHPLC as depicted in figures 2- 7.

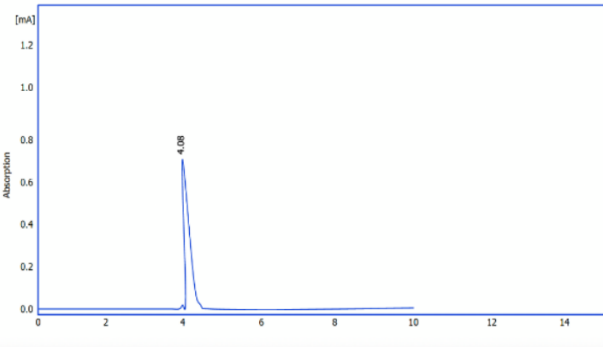


Figure (2): PHPLC chromatogram of isolated A3 compound

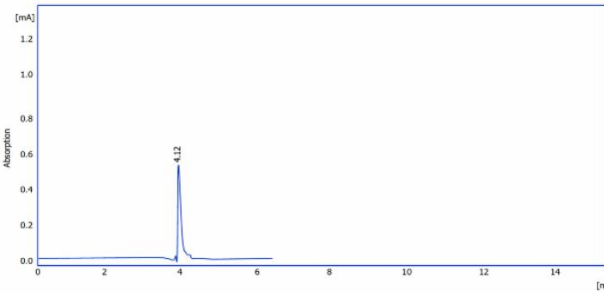


Figure (3): HPLC chromatogram of caffeic acid standard

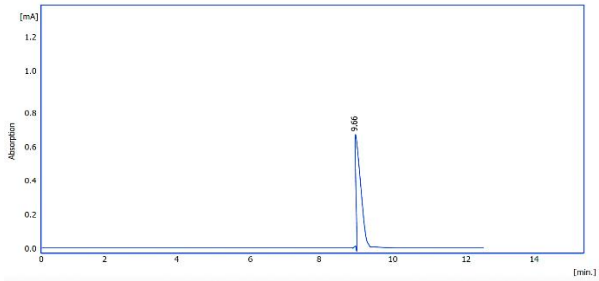


Figure (4): PHPLC chromatogram of isolated A8 compound

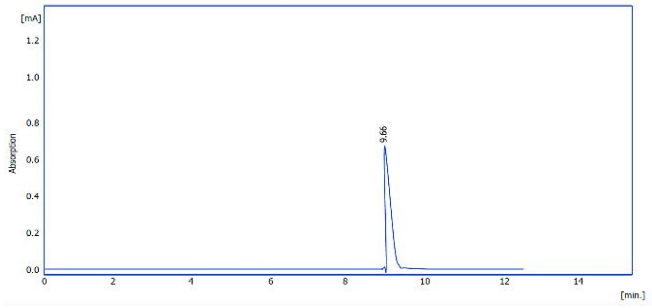
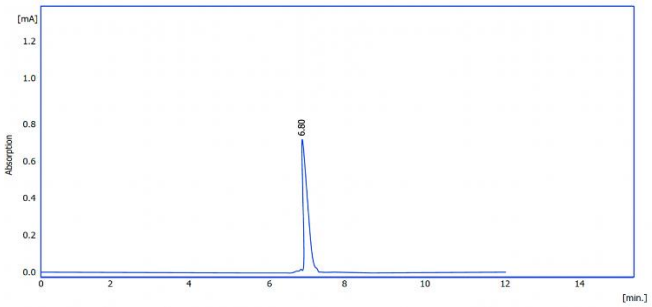


Figure (5): HPLC chromatogram of luteolin standard



Figure(6): PHPLC chromatogram of isolated A2 compound

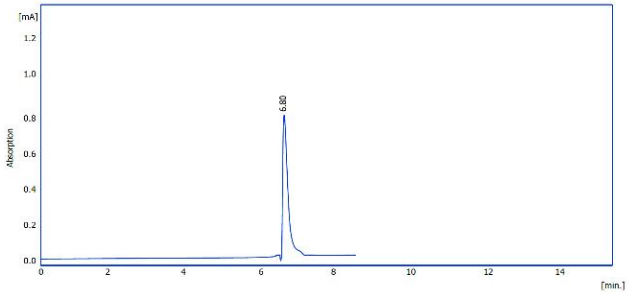


Figure (7): HPLC chromatogram of Gallic acid standard

Analysis by FT-IR

Fourier transform infrared spectroscopy analysis of Compound A3 as in figure 8, A8 as in figure 9, and IR spectra of Compound A2 in figure 10. IR spectroscopy analysis of Compound A3 revealed the presence of 3346.5 bands referring to phenolic O-H stretching band,2972 referring to C-H stretching of the benzene ring,2887 referring to asymmetric and symmetric stretching of CH2,1649 referring to aromatic C=O stretching vibration band,1454 refer to O-H bending of carboxylic acid,881 refer to C-H bending fingerprint of aromatic(out of plane) so IR

spectra of A3 matching with IR spectra of caffeic acid standard [36]. In addition, A8 IR spectra revealed the presence of the 3381 band refers to O-H stretching of phenol, the 2904 band refers to C-H stretching of phenol, the 1651 band refers to C=C stretching vibration,1166 refers to C-O-C stretching, 1029 refers to C-O-H stretching so IR spectra of A8 matching with IR spectra of luteolin standard [37]. A2 IR spectra revealed the presence336.93 band refer to the O-H stretching broad band of phenol,3284 bands refer to carboxylic acid O-H stretching band,1618 bands refer to the C=O stretching of carboxylic acid,1541.1 refers to Aromatic C=C stretching bands,1450 band refers to O-H bending of phenyl O-H,1203 and 1099 bands refer to C-H bending fingerprint of aromatic (in-plane), 867, 663 and 790 bands refer to C-H bending fingerprint of aromatic (out of plane) So IR spectra of A2 matching with IR spectra of gallic acid standards [38].

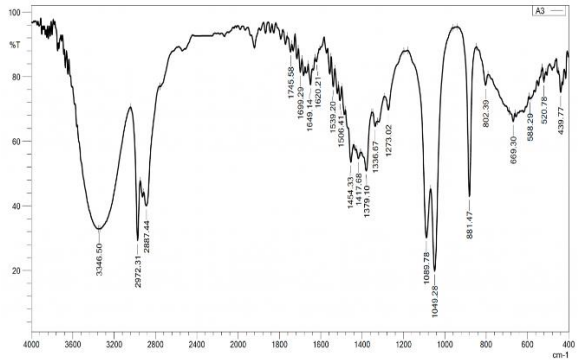


Figure (8): FTIR spectrum of isolated A3 compound

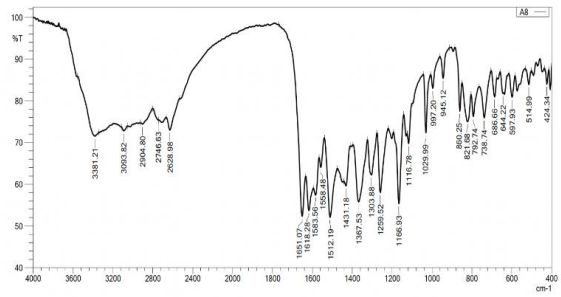


Figure (9): FTIR spectrum of isolated A8 compound

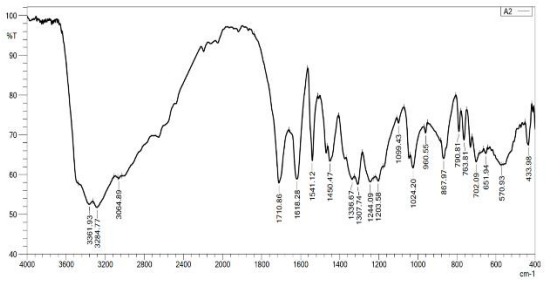


Figure (10): FTIR spectrum of isolated A2 compound

MTT assay

The anticancer effect of guava leaves F3 fraction against AMJ-13 cancer cell line was weighed by MTT assay, and AMJ-13 was exposed to sequential concentrations (31.2, 62.5,125, 250, 500, 1000µg/ml) of guava leaf F3 for 72 h to assess its effects on the viability of cell line depending on a concentration-dependent mode as shown in figure11. Guava leaf exhibited a decrease in cell viability (%) with IC_{50} values of 414.3 µg /ml as in figure 12. Also, guava leaves F3 made morphological alterations and cell loss as shown in figure 13. The anticancer effect of guava leaves F3 against the MCF-7 human cancer cell line was weighed by MTT assay.MCF-7was exposed to sequential concentrations (25 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml) of the guava extract to assess its effects on the viability of cell line as shown in figure14. The decrease in MCF7cell viability (%) by guava F3 is presented in table 1. All concentrations of guava leaf extract compared to control significantly inhibited cell lines ($P < 0.0001$). Guava F3 also made morphological alterations and cell loss as shown in figure15.

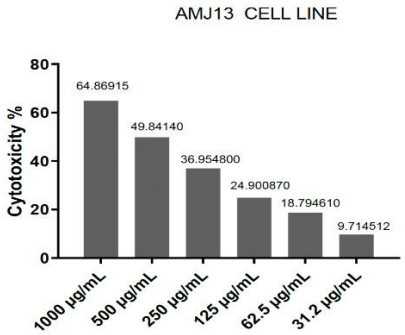


Figure (11): Cytotoxic effect of F3 fraction guava leaves on AMJ13 cell line

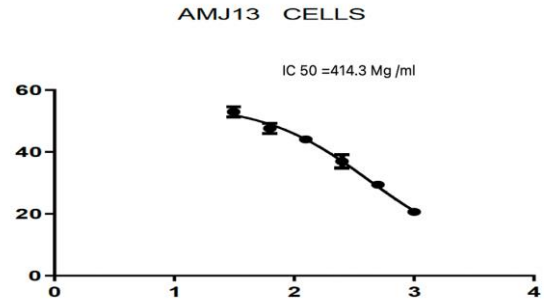


Figure (12): IC_{50} of F3 guava leaves on AMJ13 cell line

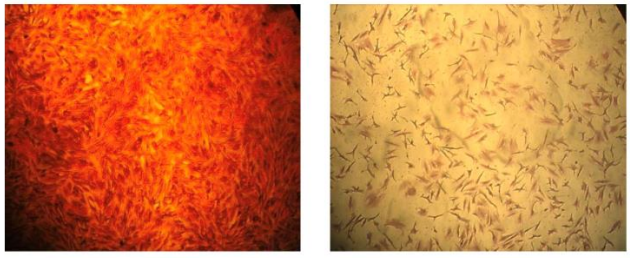


Figure (13): Morphology of untreated AMJ13(A). Morphology of AMJ13 cell line after treatment with F3 fraction of Guava leaves (B). Hematoxylin and Eosin staining, under magnification power 100x.

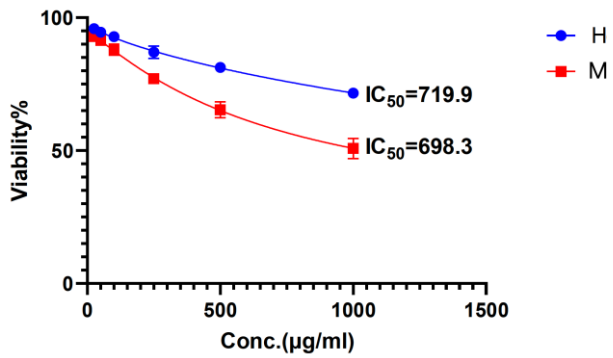
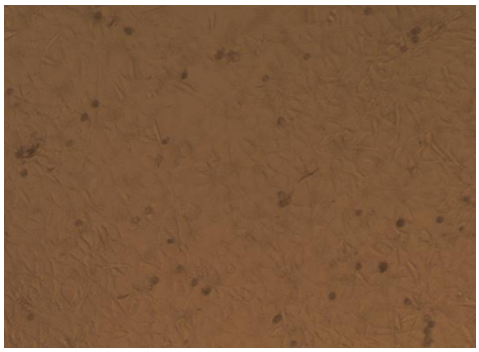
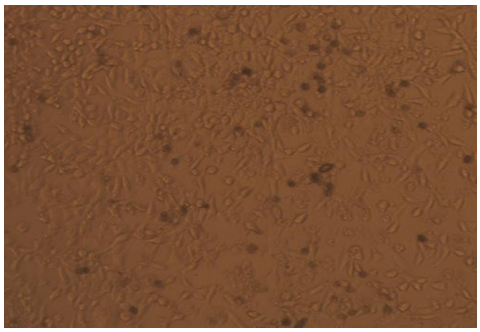


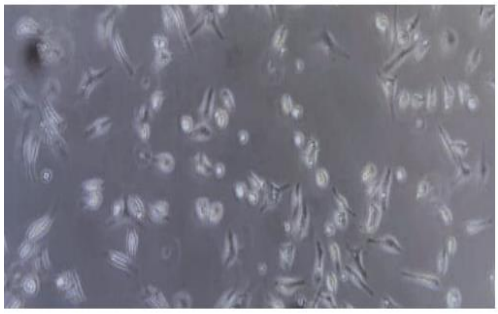
Figure (14): IC_{50} of guava F3 fraction on MCF7



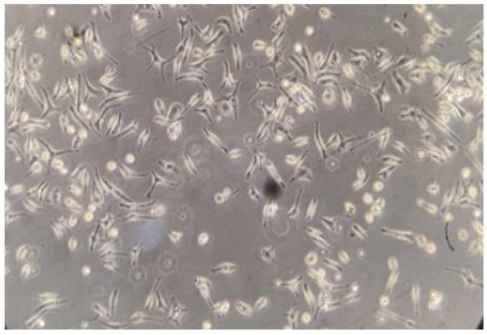
A



B



C



D

Figure (15). Morphology of control(A). Morphology of MCF7 cell line after management with guava leaves F3 at conc. 1000 µg/ml(B). Morphology of HDFn before management with guava leaves F3(C). Morphology of HDFn after treatment with guava leaves F3(D). Hematoxylin and Eosin staining, under magnification power 100x.

Table (1): Cytotoxic effect of guava leaves F3 on MCF7 and HdFn cells after 72 hours incubation at 37ºC

Guava leaves extract concentration (µg mL)	Viable cell counts of MCF7cell line (Mean ± S.D)	Viable cell counts of HdFn cell line (Mean ± S.D)
1000	50.77167±3.743934	71.64333±1.837285
500	65.39367±3.042849	81.28867±1.528013
250	77.04467±1.831309	87.037±2.311917
100	87.92467±2.087783	92.86267±1.274761
50	91.47367±1.164728	94.52167±0.98406
25	92.97833±1.226403	95.83367±0.30634

Discussion

Extraction of guava leaves

This study used an ultrasonic bath sonicator a rapid and effective extraction technique that uses ultrasound to generate rapid movement of solvents, resulting in a higher mass transfer speed and acceleration of extraction resulting in a higher percentage yield of approximately 20 % w/w from 50g of guava leaves. Compared to other traditional methods of extraction like maceration and Soxhlet this method is more economical, eco-friendly, and convenient for getting good extraction results as remembered by previous studies (39). So, this method is used for guava leaf extraction to get a higher percentage yield because better conditions are obtained, the ultrasonic waves at 40 KHz, utilizing 50g of leaf powder in 750 ml of 70% ethanol at 30°C for 40 minutes.

Preliminary phytochemical investigation

An alkaline reagent test was used as a preliminary phytochemical investigation to decide the incidence or lack of polyphenolic compounds in Iraqi *Psidium guajava* the results were positive where a yellow color appeared and it appeared colorless by adding drops of diluted HCL indicating the presence of phenolic compound in F3 and F4 fractions. These results are consistent with the results of previous studies to discover the presence of phenolic compounds by using an alkaline reagent test (40). Therefore, the results were positive because guava leaves contain a large number of phenolic compounds and many flavonoids were isolated from guava leaves such as ellagic derivatives, quercetin, resveratrol, daidzin, guaijaverin, avicularine, hyperin, morin, chlorogenic acid, rutin, vanillic acid, p-hydroxyl benzoic acid, syringic acid, myricetin, naringenin,

and apigenin as previously reported (15,16). Analysis of Guava Fractions by High-performance liquid chromatography. This analytical method is a practical way to determine the presence of a wide range of substances contained in the extract of plant leaves. The retention time of isolated compound A3 was 4.08, the retention time of the caffeic acid standard was 4.12, and retention time of the isolated compound A8 was 9.66 and the retention time of the luteolin standard was 9.66. Also, the retention time of the isolated compound A2 was 6.08, and the retention time of the gallic acid standard was 6.08 as shown in figures from 1 to 7. Retention time is the amount of time a compound spends on the column after it has been injected, if a sample contains several compounds, each compound in the sample will spend a different amount of time on the column according to its chemical composition (40). In this study, HPLC examination indicates a remarkably close retention time value of caffeic acid standard, luteolin standard, and gallic acid standard matched to the isolated A3, A8, and A2 compounds isolated by PHPLC, a single peak in HPLC chromatogram usually correlates to a single compound and thus can be used to obtain important information about individual constituents of crude extracts compared with standard compounds as shown in figures from 1 to 7.

FTIR spectra

IR spectroscopy analysis of compound A3 revealed the presence of many major peaks at 3346.5 bands referring to phenolic O-H stretching band,2972 referring to C-H stretching of the benzene, 2887 referring to asymmetric and symmetric stretching of CH2, 1649 referring to aromatic C=O stretching vibration band,1454 refer to O-H bending of carboxylic acid. Also, IR spectroscopy of the A8 compound revealed the presence of many major peaks at3381 band refers to O-H stretching of phenol, the 2904 band refers to C-H stretching of phenol, the 1651 band refers to C=C stretching vibration,1166 refers to C-O-C stretching, 1029 refers to C-O-H stretching. While IR spectroscopy of A2 showed the presence of many major peaks at 3361.93 referring to the O-H stretching broad band of phenol,3284 bands refer to carboxylic acid O-H stretching band,1618 bands refer to the C=O stretching of carboxylic acid. Compared to previous studies most of the major peaks appear the same for caffeic acid, luteolin, and gallic acid tested by FTIR (36-38). So, these functional groups present in the spectroscopy of A3, in turn, indicate the presence of phenolic acid by the presence of major peaks at 3346.5 bands referring to the phenolic O-H stretching band this indicates that this isolated compound belongs to the phenolic compounds, IR spectroscopy of A8 showed the presence of functional groups related to phenol, benzene ring and carbonyl indicate that isolated compound is flavonoid compound as compared with literature studies (37). The FTIR spectroscopic technique of A2 confirms the presence of functional groups in isolated compound A2 that were respectively related to phenol, carbonyl, and benzene, of the phenolic acid compound.

The MTT assay: It is a sensitive and reliable indicator of cellular metabolic activity and is preferred over the other methods measuring this end-point like the ATP and 3H-thymidine incorporation assay, The MTT assay relies on the reduction of MTT, a yellow water-soluble tetrazolium dye, primarily by the mitochondrial dehydrogenases, to purple colored formazan crystals (41). The MTT assay was used to determine the cytotoxicity against AMJ-13 and MCF-7 cancer cell lines, and the results showed that the F3 had a dose-dependent inhibitory activity against AMJ-13 cell growth, with cytotoxicity % of 9.7%, 18.7%, 24.9%, 36.95%, 49.8%, and 64.8% after exposure to 31.2, 62.5, 125, 250, 500 and 1000 µg /mL, respectively, all concentrations of guava leaf extract compared to control significantly inhibited cell lines ($P < 0.0001$). also showed that the F3 had a dose-dependent inhibitory activity against MCF-7 cell growth shown in table 1, a cytotoxicity test was also carried out on normal cells (HdFn cells) to determine the selectivity of the fraction, The purpose of this selectivity value is to determine the level of safety of an anticancer compound against normal cells so that it can be further developed as a chemopreventive agent, F3 fraction noncytotoxic to HDFn, with an IC50 value719.9 µg/mL substantially surpassing concentrations of 100 µg/mL(42). The parameter used for the cytotoxic test was the IC50 value indicating the concentration value that results in the inhibition of cell proliferation by 50% and the potential toxicity of a compound to cells. The IC50 value can show the potential of a compound as being cytotoxic. As the IC50 value increases, the compound toxicity decreases. The results of a cytotoxicity test on the target organ provide direct information about changes that occur specifically in cell functions. The anticancer effect of guava leaves F3 against AMJ-13 and MCF-7 cancer cell lines exhibited a decrease in cell viability (%) with IC50 values of 414.3 µg /ml against AMJ-13 and IC50 values of 698.3 µg /ml against MCF-7. Compared with previous studies the n-hexane fraction has higher activity and selectivity to cancer cells MCF-7 than the ethyl acetate fraction(F3) with IC50 4.8 µg /ml (43). Also, Abdel-Aal *et al.* (2022), showed that the cytotoxic effect of guava leaf extract on MCF7 presented by IC_{50} is 97.5 used 52, 97, 193, and 500µg/ml respectively (44). Other results indicated that the α-humulene compound isolated from the essential oil of guava leaves was active on MCF-7 cells with an IC_{50} value of 0.082 mg/mL (45). In the present investigation, the cytotoxic activity of F3 is more effective toward AMJ13 than MCF-7with cytotoxicity %64.869 at 1000 µg /ml, to the best of our knowledge, this study is the first to biologically evaluate the guava F3 using the *in vitro* cytotoxic potential of this plant toward AMJ-13 cell line, which suggests the anticancerous activity of plant extract can probably be attributed to the phenolic compounds, as determined by the MTT cytotoxicity assay. The cytotoxic activity of the guava leaf F3 against AMJ-13 and MCF-7 might be attributed to its

ability to provoke cytotoxic and apoptotic responses within cancer cells. It was observed that the extract hindered the cell cycle progression at the G1 phase, leading to cell death (46). The implicated signaling mechanisms entail the inhibition of the AKT/mammalian target of rapamycin (mTOR) ribosomal p70 S6 kinase (S6K1) and the mitogen-activated protein kinase (MAPK) activation pathways. It is noteworthy that an elevation in AKT signaling via the mTOR pathway has been documented in diverse carcinoma cell lines (47-49).

Study limitations

In this study, we presented a preliminary study for the identification of some phenolic compounds found in guava leaves and studied the effect of ethyl acetate fraction on some cancer cell lines. One of the limitations of the study is the small number of newly planted trees, which resulted in the obtaining of small quantities of leaves that did not help us to conduct further research procedures such as extracting essential oils from the leaves and knowing their effectiveness against cancer cell lines. Also, the small quantity of leaves did not allow us to isolate larger quantities of phenolic compounds and study their effects, each compound separately, against cancer cell lines.

Conclusion

Isolation of phenolic compounds (caffeic acid, luteolin, gallic acid) from guava leaves proved successful, employing an extraction method that utilized an ultrasonic bath sonicator to enhance the percentage of the yield. The identification of these phenolic compounds was carried out through techniques of HPLC and FTIR spectroscopy. The cytotoxic activity of the guava leaves F3 was evaluated on specific cell lines (AMJ-13 and MCF-7) which exhibited a decrease in cell viability (%) in a concentration-dependent mode.

Authors' Declaration: Conflicts of interest: None. We hereby confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the University of Baghdad /College of Pharmacy.

Conflict of Interest/ None
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Authors' contributions

Ashwaq T. Kareem: contributed to data gathering, analysis, practical (follow the procedure), and written parts of the study. Enas J. Kadhim gave final approval and agreement for all aspects of the study, supervision, revision, and rearrangement.

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عزل وتوصيف المركبات الفينولية في أوراق الجوافة وتقييم النشاط السام للخلايا ضد خلايا سرطان الثدي العراقي وسرطان الثدي البشري

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الخلاصة

الخلفية:Psidium guajava المعروفة باسم الجوافة، هي شجرة استوائية تُقدر بثمارها المغذية وخصائصها الطبية. هذا العضو من عائلة Myrtacease غني بالمواد الكيميائية النباتية، وهي مركبات طبيعية ذات فوائد صحية محتملة. أظهرت الدراسات أن أوراق وثمار الجوافة تمتلك أنشطة دوائية مختلفة، بما في ذلك خصائص مضادة للسرطان ضد العديد من خطوط الخلايا السرطانية.

الاهداف: تبحث هذه الدراسة في عزل المركبات الفينولية لأوراق الجوافة المزروعة في العراق واختبار النشاط السام للخلايا (القدرة على قتل الخلايا السرطانية) ضد خطين من خلايا سرطان الثدي: خط الخلايا AMJ-13 العراقي وخط الخلايا البشرية MCF7 الراسخ لجزء الاثيل اسيتيت.

الطريقه: سيستخدم الباحثون تقنية قوية تسمى التحليل اللوني السائل عالي الأداء (HPLC) ومطياف الأشعة تحت الحمراء لتحويل فورييه (FTIR) لتحليل مستخلص أوراق الجوافة ولمعرفة هيكلية المركبات. وفي وقت لاحق، سيتم استخدام HPLC التحضيري (PHPLC) تقوم هذه الطريقة بفصل المكونات المختلفة داخل المستخلص، وهو شكل متخصص من HPLC، لعزل المركبات الفينولية المحددة ذات الاهتمام.

النتائج: نجح الباحثون في عزل عينات نقية من حمض الكافيك، واللوتيولين، والكالك اسد، وجميعها مركبات فينولية من مستخلص أوراق الجوافة باستخدام HPLC التحضيري. ثم تم استخدام جزء الاثيل اسيتيت المعزولة منه المركبات الفينولية لمعرفة نشاطه السام للخلايا ضد كل من خطوط خلايا سرطان الثدي العراقية AMJ-13 وMCF7 البشرية. وأظهرت النتائج انخفاضًا في حيوية الخلية، مما يشير إلى خصائص المستخلص المحتملة المضادة للسرطان. كان المستخلص أكثر فعالية ضد خلايا AMJ-13 العراقية بقيمة IC50 البالغة 414.3 ميكروغرام / مل، مقارنة بخلايا MCF7 البشرية بقيمة IC50 البالغة 698.3 ميكروغرام / مل.

الاستنتاج: قدمت هذه التقنيات التحليلية المستخدمة في هذه الدراسة، مثل HPLC,FTIR ملفًا تفصيليًا للمركبات الفينولية الموجودة في أوراق الجوافة. تشير هذه المعلومات، بالإضافة إلى اختبارات السمية الخلوية، إلى أن أوراق الجوافة لديها القدرة على قتل الخلايا السرطانية بطريقة تعتمد على التركيز.

الكلمات المفتاحية: استخلاص بالموجات فوق الصوتية، جوافة حامض الكافييك كالك اسد,لتيولين.