AL-KUFA JOURNAL FOR BIOLOGY
ISSN [Print]: 2073-8854 **ISSN [online]:** 2311-6544

Original Research Paper

Evaluation of the Effectiveness of Dry and Fresh Aqueous Extracts of *Cordia myxa* on A549 Cell Line

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Article history
Received:21/01/2025
Revised: 16/03/2025
Accepted: 24/03/2025

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Abstract Cancer, one of the prevalent chronic illnesses affecting humans, this study aims to evaluate the anticancer potential of aqueous extracts from both dry and fresh *Cordia myxa* fruits through bioassays conducted on human lung cancer cell lines [A549]. Methodology included preparing the *Cordia myxa* aqueous extracts and maintaining A549 cell cultures in RPMI-1640 medium supplemented with 10% Fetal bovine serum, penicillin, and streptomycin. Two methods were employed to investigate the impact of *Cordia myxa*. The first method involved Gas Chromatography-Mass Spectrometry [GC-MS], which was used to analyze and compare the secondary metabolites in both extracts to determine which extract exhibits higher biological activity or efficacy. The second method involved cytotoxicity assays to determine the cytotoxic effect of the aqueous extracts on A549 cells. The results demonstrate that the relative quantitative analysis confirmed the presence of phenols and alkaloids in the dried fruits. Moreover, dried *Cordia myxa* fruits showed a highly cytotoxic effect against A549 cells and caused clear morphological changes in the cell lines after treatment. This suggests that the *Cordia myxa* plant is rich in bioactive components with anticancer, anti-inflammatory, and antioxidant properties, justifying its traditional use in medicine for treating various diseases. Conclusion: The findings revealed that the dried *Cordia myxa* fruit has ~~high~~ cytotoxic activity against the A549 cell line than the fresh extract.

Keywords: *cordai myxa, apoptosis ,GC-mass,*

1.Introduction

Cancer is a condition characterized by the uncontrolled proliferation of aberrant cells, eventually resulting in damage to the organism. Cancer is the second leading cause of death in the United States, exceeded only by heart disease [30, 27].

Cancer cells use a variety of mechanisms to improve their survival, progression, and resistance to

anticancer therapies, including disrupting growth regulatory pathways, suppressing the immune system, downregulating antigens that activate T lymphocytes, inducing anti-apoptotic signals , activating multiple DNA repair pathways , and promoting the efflux of drug from the cytoplasm [22].

Lung cancer is an extremely aggressive and common disease globally, with a predicted 2.2 million new cases and 1.8 million deaths in 24]. A549 lung cells, derived from human alveolar carcinoma, exhibit characteristics typical of type II alveolar cells while also sharing several features with primary human alveolar epithelial cells [17]. The A549 cells are characterized as hypotriploid human alveolar basal epithelial cells. They are squamous in nature and are responsible for the diffusion of materials, such as electrolytes and water, across the alveoli of the lungs [21]. .

Medicinal plants have been used for the treatment, cure, or prevention of disease for centuries, making them one of the oldest forms of medical practice due to their diverse range of organic compounds that exert specific physiological effects on the human body [12] . *Cordia myxa* fruit, referred to as "Bumber" in Iraq, belongs to the Boraginaceae family, which has 300 species known globally, mostly found in wormer areas such as Central and South America, Asia, and Africa [3]. The active metabolic compounds include phenolic compounds, flavonoids, glycosides, essential oil, sterols, terpenoids, saponins, alkaloids, tannins, coumarins, gums, resins, xanthense, and mucilage [4].

Phenolic compounds in plants, along with a variety of other endogenous metabolites, operate as free radical scavengers with potent antioxidant activity. This demonstrates their ability to neutralize, eliminate free radicals, and prevent diseases associated with oxidative stress [25]. In the human body, cellular processes eventually generate hazardous molecules, including reactive oxygen species and free radicals, which may damage living cells and cause a variety of clinical diseases. [5]. Phenolic acids are known for their potential to enhance bile secretion, reduce blood cholesterol and lipid levels, and demonstrate antimicrobial, anti-inflammatory, antioxidant, cytotoxic, and anticancer properties [26]. Different parts of the plant, such as the fruit, bark, leaves, and seeds, are utilized for their antibacterial, antifungal, antidiabetic, anticancer properties, asthma, tooth decay and bronchitis. These uses promise efficacy with fewer side effects [19].

2. Methodology

Plant Collection

Cordia myxa fruits were collected from *C. myxa* trees available in al-Najaf governorate gardens, Iraq, during the period from July to August 2024. The fruits were washed and checked carefully, and any that were physically damaged or showed signs of microbial contamination were excluded.

Extraction Procedure of dry Cordia myxa

The plant extraction procedure of Al-Hamdani *et al*.,2019 with some modifications, was performed. The harvested fruits were properly washed with tap water followed by deionized water. The seeds were removed manually, and the eatable parts of the crop were collected, dried in the shade, and ground into a fine powder using an electric grinder. 40 g of dried *C. myxa* powder was placed in a conical flask and dissolved in 400 ml of distilled water. The mixture was stirred on a magnetic stirrer hotplate for 1 hour to ensure proper mixing and then placed in a cooling centrifuge at 4500 rpm at 4 C°. On the second day, the solution was first filtered through medical gauze, followed by filtration with filter paper. The resulting aqueous extract was then placed in an oven at 40C° to dry. Finally, the crude extract was collected and stored at 4 C° until use [2].

Extraction Procedure of fresh Cordia myxa

The plant extraction procedure, based on [14] with some modifications, was performed. The harvested fruits were thoroughly washed, first with tap water, then with deionized water. The seeds were manually removed. Subsequently, 40 g of fresh *Cordia myxa* fruits were placed in a glass beaker containing 400 ml of distilled water and blended using an electric blender for 15 minutes to ensure proper homogenization. The extract was collected, filtered through medical gauze, and centrifuged at 3500 rpm for 10 minutes. The resulting aqueous extract was dried in an oven at 40 C° until completely dry and stored at 4 C° until used [Harborne, 1998].

The chemical analysis of plant extracts using GC-MS

The gas chromatography–mass spectrometry [GC-MS] analysis of the aqueous extracts from both dry and fresh *Cordia myxa* fruits was performed using a Shimadzu 15A instrument. The capillary column used was a DB-5 [50 m × 0.2 mm, film thickness 0.32 μm]. The initial column temperature was set at 60 °C for three minutes, then increased to 220 °C at a rate of 5 °C/min and held constant at 220 °C for five minutes. The split/splitless injector temperature was 250 °C, and the flame ionization detector [FID] was also set at 250 °C. A 1 μL sample of the plant extract was injected, and nitrogen gas was used as the carrier gas at a flow rate of 1 mL/min. The GC-MS analysis should specify how peak identification was confirmed were identified by

comparing their spectra to those in the Wiley and NIST mass spectral libraries.

Maintenance of cell cultures

A549 cell line were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37 °C [Al-Ziaydi, 2020].

Cytotoxicity Assays

To determine the cytotoxic effect of aqueous extracts from both dry and fresh *Cordia myxa* fruits, the MTT assay was done using 96-well plates [7, 9]. Cell line were seeded at 1×10^4cells/well. After 24 hrs. Or a confluent monolayer was achieved, It's better to use a normal lung cell line as control than MCF10A because MCF10A is from a different tissue origin, so its response may not accurately represent normal lung cell behavior.

cells were treated with aqueous extracts from both dry and fresh *Cordia myxa* fruits. Cell viability was measured after 24, and 48 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO [Dimethyl Sulphoxide] followed by 37 °C incubation for 15 min with shaking 5]. The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth [the percentage of cytotoxicity] was calculated as the following equation [8].

Inhibition rate = A- B/A*100

where A is the optical density of control, and B is the optical density of the samples [8].

To visualize the shape of the cells under an inverted microscope, the cell were seeded into 24-well micro-titration plates at a density of 1×10^5 cells mL−1 and incubated for 24 h at 37 °C. Then, cells were exposed to aqueous extracts for 24hr. After the exposure time , the plates were stained with crystal violet stain and incubated at 37 °C for 10–15 min [6]. The stain was washed off gently with tap water until the dye was completely removed. The cells were observed under an inverted microscope at 100× magnification and the images were captured with a digital camera attached to the microscope [9].

Cytotoxicity = A-B/A *100

Where A and B are the optical density of control and the optical density of test

3. Results and discussion

Studies have shown that traditional medicine based on plant extracts is more cost-effective, clinically efficient, and generally associated with fewer side effects compared to modern pharmaceuticals. The secondary metabolites produced in plants demonstrate a range of biological activities advantageous to humans, including anticancer, anti-inflammatory, and antioxidant properties [Tran *et al*., 2020] .

The chemical compounds of the plant extract were characterized using GC-MS technology Table [1] and Fig [1]—an effective technique with high specificity and sensitivity used in various applications.

Table 1. Shows the result of GC-MS analysis revealed 11 main phytochemical compounds in the aqueous extract of *Cordia myxa* dry fruit

No.	RT [min]	Area %	Name	Quality	CAS Number
1	**28.998**	**15.59**	**Octadecanoic acid, methyl ester**	**97**	**000112-61-8**
2	**34.357**	**12.76**	**Bis[2-ethylhexyl] phthalate**	**50**	**000117-81-7**
3	**22.024**	**12.06**	**Gentisic acid**	**53**	**003618-20-0**
4	**18.760**	**11.98**	**2-methyl-4-[[4-methylphenyl]sulfonyl]-5-nitroimidazole**	**32**	**113121-75-8**
5	**24.852**	**9.21**	**Cyclononasiloxane, octadecamethyl-**	**72**	**000556-71-8**
6	**25.910**	**8.44**	**Palmitic acid, methyl ester**	**97**	**000112-39-0**

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7	**34.928**	**7.10**	**N[a]-Ethyl-2,16-dihydro-15.beta.-[1-oxopropyl]deethylibophyllidine**	**22**	**000000-00-0**
8	**27.394**	**5.00**	**Bistrimethylsilyl n-acetyl eicosasphinga-4,11-dienine**	**50**	**000000-00-0**
9	**15.118**	**4.63**	**Dodecamethylcyclohexasiloxane**	**76**	**000540-97-6**
10	**38.379**	**4.60**	**Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-**	**53**	**000000-00-0**
11	**41.087**	**3.14**	**Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-**	**46**	**019095-24-0**

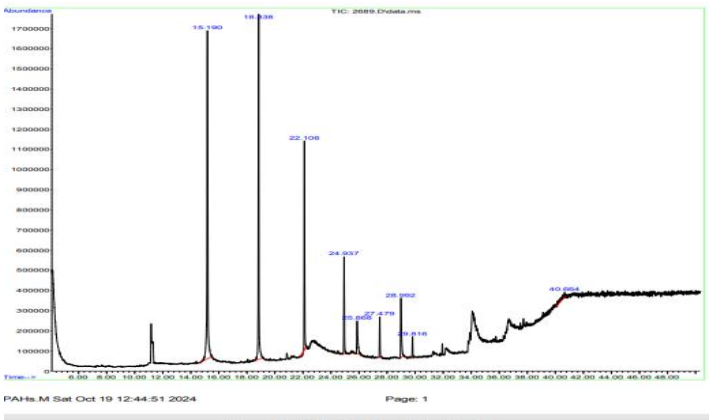

Fig 1. Shows the GC-mass analysis of dry aqueous extract.

The chemical compound of dry extract were as [Octadecanoic acid, methyl ester [1], Bis[2-ethylhexyl] phthalate [2], Gentisic acid [3], 2-methyl-4-[[4-methylphenyl]sulfonyl]-5 nitroimidazole[4],Cyclononasiloxane, octadecamethyl- [5], Palmitic acid, methyl ester [6], N[a]-Ethyl-2,16-dihydro-15.beta.-[1- oxopropyl] deethylibophyllidine [7], Bistrimethylsilyl n-acetyl eicosasphinga-4,11-dienine [8], Dodecamethylcyclohexasiloxane [9], Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- [10],Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- [11], and these compounds are displayed in the GC-MS scheme [Figure 1].

Table 2. Shows [9] phytochemical compounds were identified in the aqueous extract of fresh ***Cordia myxa*** fruit

No.	RT [min]	Area%	Name	Quality	CAS Number
1	**15.191**	**29.72**	**Cyclohexasiloxane, dodecamethyl-**	**93**	**000540-97-6**
2	**18.838**	**21.35**	**Cycloheptasiloxane, tetradecamethyl-**	**50**	**071579-69-6**
3	**22.107**	**10.36**	**Cyclooctasiloxane, hexadecamethyl-**	**46**	**056114-62-6**
4	**28.992**	**7.92**	**Methyl stearate**	**99**	**000112-61-8**
5	**24.935**	**5.34**	**Cyclononasiloxane, octadecamethyl-**	**90**	**000556-71-8**
6	**25.869**	**3.61**	**Hexadecanoic acid, methyl ester**	**98**	**000112-39-0**
7	**27.477**	**1.98**	**Cyclononasiloxane, octadecamethyl-**	**50**	**000556-71-8**
8	**40.662**	**1.36**	**Octasiloxane, - hexadecamethyl-**	**90**	**019095-24-0**

9	**29.817**	**1.10**	**Cyclononasiloxane, octadecamethyl-**	**38**	**000556-71-8**

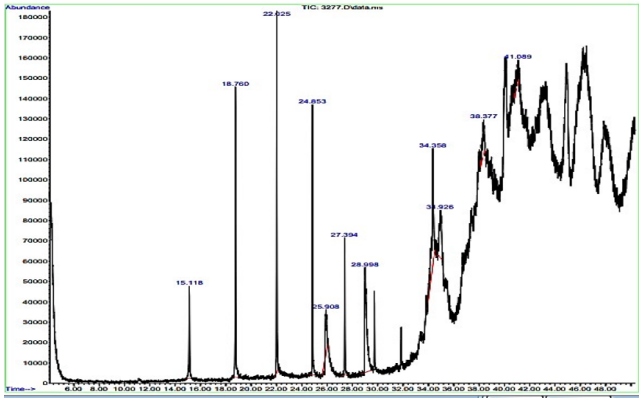

Fig 2. Shows the GC-mass analysis of wet aqueous extract

The chemical compound were as Cyclohexasiloxane ,dodecamethyl- [1], Cycloheptasiloxane, tetradecamethyl- [2], Cyclooctasiloxane hexadecamethyl- [3], Methyl stearate [4], Cyclononasiloxae, octadecamethyl- [5], Hexadecanoic acid, methyl ester [6], Octasiloxane, - hexadecamethyl- [7] , these compounds are displayed in the GC-MS scheme [Fig 2]. The identification of active chemical compounds was dependent on retention time, peak area percentage, and mass spectral fragmentation patterns, compared to known compounds in the National Institute of Standards and Technology [NIST] and Wiley libraries.

The aqueous extract from dried *Cordia myxa* fruits contained more chemical compounds and produced better results than the extract from fresh fruits, according to a comparison. The analysis revealed that dried fruits contain gentisic acid, a type of phenolic compound that acts as a free radical scavenger with strong antioxidant activity. Previous studies of the *C. dichotoma* methanol extract from the leaves have demonstrated that phenolic compounds possess significant biological properties, such as powerful antioxidant effects, anticancer activities, and the ability to eliminate free radicals [25]. A study revealed that methanolic extracts from the fruits of *Cordia dichotoma Forst* and *Cordia myxa L* contain the highest levels of phenols. This indicates that the flavonoids and phenolic compounds present in both samples collectively enhance their antioxidant potential[33].

Dried fruit was also found to contain octadecenoic acid, methyl ester, which represents the highest proportion among the compounds found in the dried extract, accounting for 15.59%. According to previous studies, this compound is associated with various pharmacological activities, including anti-cancer, anti-inflammatory, antiandrogenic, hypocholesterolemic, anemiagenic, and cancer prevention properties[Reddy *et al*., 2020]. Moreover, the dried fruit contains N[a]-Ethyl-2,16-dihydro-15β-[1-oxopropyl] deethylibophyllidine, a type of alkaloid. A study by[4] investigated the beneficial effects of alkaloids present in the solvent extract of *Cordia myxa* leaves against certain pathogenic bacteria. Their results indicate that this extract could serve as a natural therapeutic agent for treating infections and inflammations, providing a safer alternative to synthetic drugs, which are often associated with side effect[s4]. Palmitic acid [also known as hexadecanoic acid] is one of the compounds present in dried and fresh fruits. Quantitative analysis revealed that the relative content of hexadecanoic acid was 8.44% in dried fruit, compared to 3.61% in fresh fruit. Raina *et al*. studied the bark of *Cordia dichotoma*, showing the presence of active chemicals such as hexadecanoic acid and investigating its pharmacological potential, including its anti-cancer and anti-inflammatory properties [28]. Previous research has shown that hexadecanoic acid has a lot of potential as a drug. It has anticancer, anti-inflammatory, hypocholesterolemic, antispasmodic, antiviral, and nematode-killing properties [29].

The current results are consistent with previous research on the main bioactive chemicals found in *Cordia myxa* fruit. Larki *et al*. analysed extracts from fresh and dried *Cordia myxa* fruits, finding that extracts from dried fruits had better results compared to those from fresh fruits [23].

The cytotoxic effect of aqueous extracts from both dry and fresh *Cordia myxa* fruits on the A549 cell line was studied. The anti-proliferative activity of the aqueous extracts was evaluated by their ability to inhibit cell proliferation. The results demonstrated that the dry *Cordia myxa* fruits had a highly cytotoxic effect on the A549 cell line, as shown in Figures [3-4].

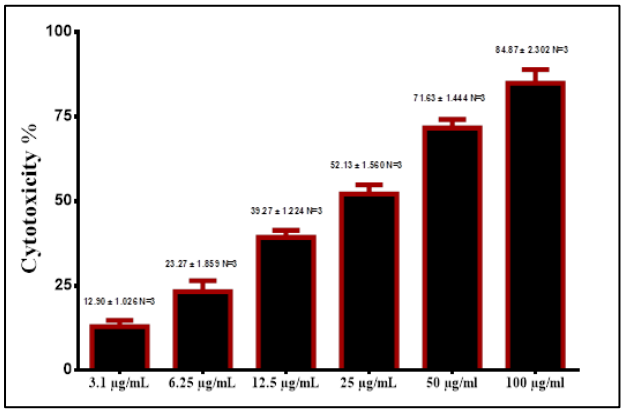

Fig 3. Cytotoxicity effect of [DRY EXTRACT] in A549 cells. IC50=18.65 µg/ml

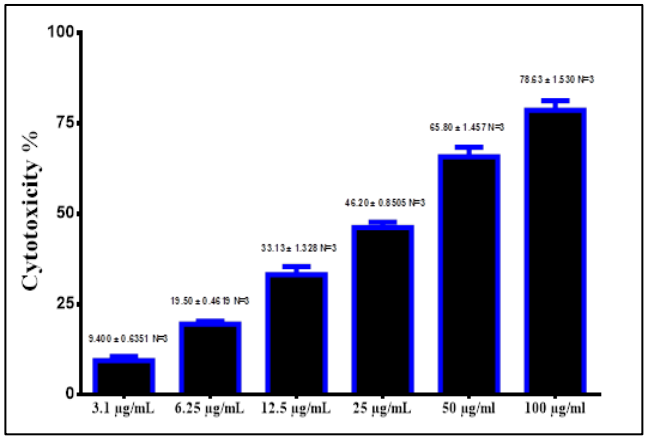

Fig 4. Cytotoxicity effect of [WET EXTRACT] in A549 cells. IC50=24.58 µg/ml

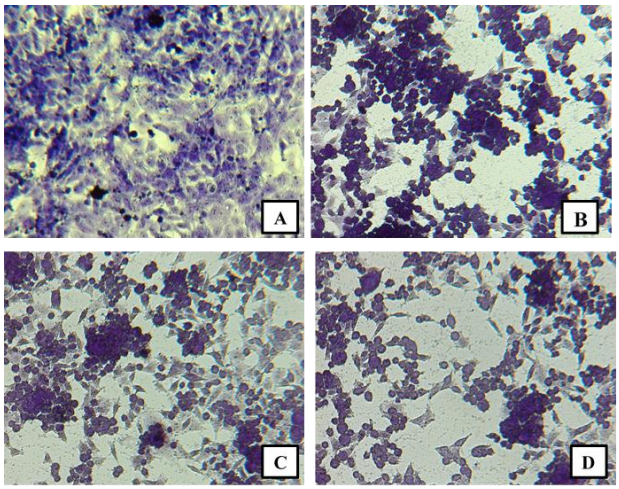

Fig 5. A549 cells section in controls and treated with different concentration by *cordia myxa* dry aqueous extract. A: Control untreated. B: After treated with 12.5 µg/ml. C: After treated with 50 µg/ml. D: After treated with 100 µg/ml. Magnification power 10x

The MTT assay technique was employed to evaluate the cytotoxic effects of both dry and fresh *Cordia myxa* fruit extracts at the IC50 concentration on the A549 cell line. The results revealed that the dry fruit extract exhibited significant anti-proliferative activity, effectively inhibiting cell growth in a dose-dependent manner. Furthermore, the dry *Cordia myxa* extract demonstrated a strong cytotoxic effect against the A549 cell line while sparing normal human cells. In addition, clear morphological changes were observed in A549 cells after treatment with the dry fruit extract, as shown in[figures -5] The sensitivity of A549 cells to different concentrations of the dry extract The concentrations were [12.5 µg/ml , 50 µg/ml and 100 µg/ml] was compared to untreated control cells. A marked reduction in cell viability was observed, with maximum inhibition at a concentration of 100 µg/ml The inhibition ratio of A549 cells increased as the concentration of the dry *Cordia myxa* extract increased.

Previous research studied the cytotoxic effects of *Cordia myxa* nano extract on A549 lung cancer cells. The findings indicated that the nano extract treatment greatly inhibited the proliferation of A549 cells in a dose-dependent way. The ability of the nano extract to destroy A549 cell lines was determined to be greater than 75% [15].

Researchers studied the bark of *Cordia dichotoma* and found that it killed A-549 human lung cancer cells in a way that depended on the dose. It suggests that the bark of *C. dichotoma* could be a significant natural source for anticancer drugs [28].

A study was conducted on the ethanol extract of *Cordia myxa* fruits, which demonstrated cytotoxic activity against breast cancer [MCF-7] cells. With an IC50 value, the extract significantly reduced cell viability to 70% at a specific concentration in a dose-dependent manner **[**1].

A study revealed that *Cordia myxa* fruit extract exhibits moderate cytotoxicity against HepG-2 liver cancer cells, with selective targeting sparing normal THLE2 cells. Although its encapsulated forms showed reduced cytotoxicity and are not as potent as cisplatin, CMF extract could serve as a safer alternative with selective anti-cancer properties [130

Researchers are actively studying plant-based products as promising anticancer therapies due to their remarkable potential in treating various diseases, including cancer. Phytochemicals, which are extracts from plant parts like fruits, seeds, roots, and barks, have the ability to stop tumour growth, angiogenesis, and metastasis while being non-toxic and free from adverse side effects [33] .

Conclusion

The dried *Cordia myxa* fruit has high cytotoxic activity against the A549 cell line than the fresh extract **.**

Author's Contributions

This work has been accomplished through the equal efforts of all researchers involved .

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017).

References

1- AL-Dallee, Z. T., Khalaf, K. T., & Atwan, Z. W. (2021). Ethanol extract of Cordia myxa L. fruit increases expression of antioxidant enzymes and tumor suppressor PTEN genes in MCF-7 breast cancer cells. *International Journal of Pharmaceutical Research, 13*(2).

2- Al-Hamdani, H. M. S., Alfraji, A. S. A., Muhammed, A. I., & Mosa, R. J. (2019). Exploration of the effect of higher doses of aqueous and alcoholic extracts of bamber fruit (Cordia myxa) on some histological parameters and symptoms in mice. *The Iraqi Journal of Agricultural Science, 50*(4), 1405-1415.

3- Al-Khafaji, S. A., Alsaadawi, M. A., Al-Yasari, A. M., & Al-Saadawe, M. A. (2021). Article review: Cordia myxa L.: The gift of nature. *Basrah Journal of Agricultural Sciences, 34*(2), 267-277.

4- Al-Maliki, A. D., Aboud, M. N., & Hameed, M. F. (2021, November). Estimation and evaluation of medicinal efficacy of maximum inhibitory concentration of alkaloids extracted from Iraqi Cordia myxa leaves against some pathogenic bacteria. In *Journal of Physics: Conference Series* (Vol. 2063, No. 1, p. 012020). IOP Publishing.

5- Al-Musawi, M. H., Ibrahim, K. M., & Albukhaty, S. (2022). In vitro study of antioxidant, antibacterial, and cytotoxicity properties of Cordia myxa fruit extract. *Iranian Journal of Microbiology, 14*(1), 97.

6- Al-Musawi, S., Albukhaty, S., Al-Karagoly, H., Sulaiman, G. M., Jabir, M. S., & Naderi-Manesh, H. (2020). Dextran-coated superparamagnetic nanoparticles modified with folate for targeted drug delivery of camptothecin. *Advances in Natural Sciences: Nanoscience and Nanotechnology, 11*(4), 045009.

7- Al Salman, H. N. K., Ali, E. T., Jabir, M., Sulaiman, G. M., & Al Jadaan, S. A. (2020). 2-Benzhydrylsulfinyl N-hydroxyacetamide Na extracted from fig as a novel cytotoxic and apoptosis inducer in A549 and AMJ 13 cell lines via P53 and caspase 8 pathway. *European Food Research and Technology*.

8- Al-Shammari, A. M., Al-Saadi, H., Al-Shammari, S. M., & Jabir, M. S. (2020). Galangin enhances gold nanoparticles as anti-tumor agents against ovarian cancer cells. In *AIP Conference Proceedings* (Vol. 2213, No. 1, p. 020206). AIP Publishing LLC.

9- Al-Ziaydi, A. G., Al-Shammari, A. M., Hamzah, M. I., & Jabir, M. S. (2020). Hexokinase inhibition using D-mannoheptulose enhances oncolytic Newcastle disease virus-mediated killing of breast cancer cells. *Cancer Cell International, 20*(1), 1-10.

10- Al-Ziaydi, A. G., Hamzah, M. I., Al-Shammari, A. M., Kadhim, H. S., & Jabir, M. S. (2020, December). The anti-proliferative activity of D-mannoheptulose against breast cancer cell line through glycolysis inhibition. In *AIP Conference Proceedings* (Vol. 2307, No. 1, p. 020023). AIP Publishing LLC.

11- Al-Ziaydi, A. G., Al-Shammari, A. M., Hamzah, M. I., Kadhim, H. S., & Jabir, M. S. (2020). Newcastle disease virus suppresses glycolysis pathway and induces breast cancer cell death. *VirusDisease*, 1-8.

12- De Moraes Mello Boccolini, P., & Siqueira Boccolini, C. (2020). Prevalence of complementary and alternative medicine (CAM) use in Brazil. *BMC Complementary Medicine and Therapies*, *20*, 1-10.

13- El-Massry, K. F., Farouk, A., Mahmoud, K. F., El-Ghorab, A. H., Musa, A., Mostafa, E. M., ... & Abdelgawad, M. A. (2021). Chemical characteristics and targeted encapsulated Cordia myxa fruit extracts nanoparticles for antioxidant and cytotoxicity potentials. *Saudi Journal of Biological Sciences*, *28*(9), 5349-5358.

14- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.

15- Hasoon, B. A., Abdulwahab, A. I., Maeh, R. K., & Al-Azawi, K. F. (2021). Preparation and characterization of silver nanoparticles by Cordia myxa extract and their study of anticancer, antioxidant, and antibacterial activity. *Indian Journal of Forensic Medicine & Toxicology*, *15*(3), 5458-5465.

16- Ibrahim, A. A., Kareem, M. M., Al-Noor, T. H., Al-Muhimeed, T., AlObaid, A. A., Albukhaty, S., & Sahib, U. I. (2021). Pt(II)-thiocarbohydrazone complex as a cytotoxic agent and apoptosis inducer in Caov-3 and HT-29 cells through the P53 and caspase-8 pathways. *Pharmaceuticals*, *14*(6), 509.

17- İslam, İ., Karagöz, A., Aytuğ, H., Bulduk, İ., & Çoban, F. K. (2022). Apoptotic and antiangiogenic effects of noscapine in human lung cancer (A549) cells. *Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi*, *9*(1), 42-49.

18- Jabir, M. S., Abood, N. A., Jawad, M. H., Öztürk, K., Kadhim, H., Albukhaty, S., ... & Sulaiman, G. M. (2022). Gold nanoparticles loaded with TNF-α and CALNN peptide as a drug delivery system and promising therapeutic agent for breast cancer cells. *Materials Technology*.

19- Javadi, M., Molavynejad, S., Saberipoor, B., Deilami, A. A., Siahpoosh, A., Mousavi, S. A., & Kambo, M. S. (2024). The effect of Cordia myxa mouthwash on the incidence and severity of stomatitis in leukemia patients undergoing chemotherapy: A protocol study. *Jundishapur Journal of Chronic Disease Care*, *14*(1).

20- Jawad, M., Öztürk, K., & Jabir, M. S. (2021). TNF-α loaded on gold nanoparticles as a promising drug delivery system against the proliferation of breast cancer cells. *Materials Today: Proceedings*, *42*, 3057-3061.

21- Korrodi-Gregorio, L., Soto-Cerrato, V., Vitorino, R., Fardilha, M., & Perez-Tomas, R. (2016). From proteomic analysis to potential therapeutic targets: Functional profile of two lung cancer cell lines, A549 and SW900, widely

studied in pre-clinical research. *PLoS One, 11*(11), e0165973.

22- Kryczka, J., Kryczka, J., Czarnecka-Chrebelska, K. H., & Brzeziańska-Lasota, E. (2021). Molecular mechanisms of chemoresistance induced by cisplatin in NSCLC cancer therapy. *International Journal of Molecular Sciences, 22*(16), 8885.

23- Larki, A., Shakiba Maram, N., Zarei Ahmady, A., Mohtasham, N., Mafakher, L., Khelghati, N., & Hedayati, E. (2020). Comparing different extraction methods for oral syrup formulation of major bioactive compounds from Cordia myxa fruit. *Eurasian Chem Commun, 2*(9), 953-960.

24- Leiter, A., Veluswamy, R. R., & Wisnivesky, J. P. (2023). The global burden of lung cancer: Current status and future trends. *Nature Reviews Clinical Oncology, 20*(9), 624-639.

25- Madhubala, M., & Santhi, G. (2019). Phytochemical and GC-MS analysis on leaves of selected medicinal plants in Boraginaceae family Cordia dichotoma L. *Pramana Research Journal, 9*, 2249-2276.

26- Mohite, A. K., & Shingare, R. M. (2020). Phytochemical analysis and ethnobotanical study of wild medicinal plants. *IJRAR-International Journal of Research and Analytical Reviews, 7*(1), 1-9.

27- Muskan Tewari. (2022). Cancer and its treatment: An overview. *International Journal of Advanced Research, 10*(03), 950-956.

28- Raina, S., Sharma, V., Sheikh, Z. N., Kour, N., Singh, S. K., Zari, A., ... & Hakeem, K. R. (2022). Anticancer activity of Cordia dichotoma against a panel of human cancer cell lines and their phytochemical profiling via HPLC and GCMS. *Molecules, 27*(7), 2185.

29- Reddy, G. J., Reddy, K. B., & Reddy, G. S. (2020). GC-MS analysis and in-vitro anti-diabetic activity of bioactive fractions of Feronia elephantum fruit. *International Journal of Pharmaceutical Sciences and Research, 11*(5), 1000-1010.

30- Rock, C. L., Thomson, C., Gansler, T., Gapstur, S. M., McCullough, M. L., Patel, A. V., ... & Doyle, C. (2020). American Cancer Society guideline for diet and physical activity for cancer prevention. *CA: A Cancer Journal for Clinicians, 70*(4), 245-271.

31- Tran, N., Pham, B., & Le, L. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology, 9*(9), 252.

32- Usmani, S., Ahmad, M., Hussain, A., Arshad, M., & Ali, M. (2018). Cellular oxidative stress and antiproliferative effects of Cordia dichotoma (Linn.) seeds extract and their fractions on human cervix epitheloid (HeLa) and human lung (A549) carcinoma cells. *European Journal of Integrative Medicine, 21*, 1-10.

33- Zahra, K., Khan, A. S., Saeed, A., & Gul, H. (2024). Studies on analysis of minerals, phytochemicals, proximate composition, and in vitro biological activities of methanolic extracts from

the fruits of Cordia dichotoma Forst and Cordia myxa L. *Biological Sciences, 4*(2), 575-584.