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Identification of bioactive compounds in *Cordia myxa* L. extract to extend the shelf life of refrigerated beef

Identificación de compuestos bioactivos en el extracto de *Cordia myxa* L. para prolongar la vida útil de la carne de res refrigerada

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ABSTRACT

This paper examined alcoholic extract of *Cordia myxa* fruits (CMF) and identified the existence of bioactive compounds, including alkaloids, saponins, flavonoids, and steroids. CMF was found to have good antioxidants, antibacterial and antifungal effects. Another purpose of the research was to examine its efficacy as a natural meat preservative and to learn the ideal concentration in order to achieve antimicrobial activity. Beef samples that were treated with 1, 3 and 5% CMF extract and kept in refrigerator at 4 °C temperature acquired evident antimicrobial effects on *Staphylococcus aureus, Salmonella Enteritidis, Pseudomonas aeruginosa* and *Escherichia coli*. The concentration of 5% produced the strongest decrease in the growth of bacteria. This paper incorporated the concept of molecular docking modeling to analyze the highly competent HPLC analysis and the element of effectiveness in inhibition of keyanti bacterial (3HWW, 1D0V, 2RKZ, 2Q0I). P-coumaric acid exhibited huge binding affinities to all target proteins. These outcomes demonstrated that these compounds have appropriate physicochemical characteristics and high biological availability, in line with the Lipinski Rule of Five. The results presented imply that these compounds can be used as the potential antimicrobial agents and food preservatives, provided that their structure is optimized. As such, *Cordia myxa* extract is a potential ingredient that can be used to preserve food as well as increase shelf life of food products in a natural and safe manner.

Palabras clave:
Actividad antioxidante
Conservación de alimentos
Calidad de la carne
Crecimiento microbiano
Compuestos fenólicos

RESUMEN

En este estudio se evaluó el extracto alcohólic de frutos de *Cordia myxa* (CMF), revelando la presencia de compuestos bioactivos como alcaloides, saponinas, flavonoides y esteroides. CMF mostró fuertes propiedades antioxidantes, antibacterianas y antifúngicas. La investigación también tuvo como objetivo evaluar su eficacia como conservante natural de la carne y determinar la concentración óptima para la actividad antimicrobiana. Las muestras de carne de res tratadas con extracto de CMF al 1, 3 y 5% y almacenadas a 4 °C durante 8 días mostraron efectos antimicrobianos significativos contra *Staphylococcus aureus, Salmonella Enteritidis, Pseudomonas aeruginosa* y *Escherichia coli*. La concentración del 5% produjo la mayor reducción en el crecimiento bacteriano. El acoplamiento molecular se realizó utilizando compuestos analizados por HPLC dirigidos a enzimas bacterianas (3HWW, 1D0V, 2RKZ, 2Q0L). El ácido P-cumárico mostró fuertes afinidades de unión con todas las proteínas diana. Los resultados del acoplamiento confirmaron la idoneidad fisicoquímica y la alta biodisponibilidad de estos compuestos, cumpliendo con la Regla de Cinco de Lipinski. Estos hallazgos sugieren que el derivado del ácido hidroxibenzoico, si se optimiza estructuralmente, puede servir como posibles agentes antimicrobianos y conservantes de alimentos. Por lo tanto, el extracto de *Cordia myxa* se presenta como una alternativa prometedora para la conservación de alimentos y la extensión de su vida útil de forma natural y segura.

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Since ancient times, humans have cultivated and utilized various plants for their medicinal properties. *Cordia myxa*, known locally as "Bumber," is one such plant with historical use dating back to the Roman era (2nd–3rd century A.D.). The genus *Cordia*, part of the Boraginaceae family The *Cordia* genus comprises approximately 300 species, including several species such as *C. dichotoma*, *C. myxa*, *C. africana*, *C. latifolia*, and *C. abyssinica* and others. The plant is edible and traditionally consumed as food, with the fruit being particularly rich in polysaccharides. It produces a sticky, jelly-like substance that serves as a natural source of antioxidants commonly used in daily life (Hussein et al. 2023).

Cordia myxa fruit has been widely used as an anthelmintic, diuretic, astringent, demulcent, and expectorant). It is also applied in treating diarrhea, malaria, chest and urinary infections, and for wound healing. Additionally, it exhibits anti-inflammatory, analgesic, antipyretic, and antiarthritic properties. Traditionally, *C. myxa* has been used for wound healing, hepatoprotective gastroprotective (Wani et al. 2024), and antileishmanial effects. These properties are attributed to their rich content of phenolic and flavonoid compounds, known for their antioxidant activity, based on its bioactive potential.

Throughout human evolution, beef has remained a vital part of the diet, valued for its high-quality protein and essential nutrients. However, meat spoilage is largely caused by microorganisms originating from the animal's skin and digestive tract. Factors such as animal husbandry, age, slaughtering conditions, processing, storage temperature, preservation methods, packaging, and consumer handling all influence microbial contamination. Meat supports diverse microflora, including both harmless and pathogenic species, making it an excellent growth medium for microbes. Microbial growth causes spoilage resulting in huge financial losses within the meat industry. Due to this, there has been a growing interest in natural preservation techniques which are capable of extending the shelf life without compromising quality and sensory attributes of meat (Pathania et al. 2010).

Treating meat with alcoholic extracts of *C. myxa* is one of such approaches to improve microbiological safety of meat. Such a mixture enhances the quality of meat and prevents microbial growth. It has been demonstrated that microbial growth of refrigerated beef over a period of time is higher than in the absence of *C. myxa* extract, which suppresses the growth of bacteria as compared to untreated samples (Kargiotou et al. 2011).

This research aims to understand how *C. myxa* extract can be used as a natural food additive in microbial growth reduction and the extension of the shelf life of meat by the inhibition of microbes responsible in meat spoilage by the bioactive compounds contained in the extract. In a bid to explore the antimicrobial prospects of *Cordia myxa* extract and its potential in alleviating meat spoilage, the molecular docking approaches were used. It is a technique to predict the interaction of bioactive compounds with target microbial enzymes, which gives information on their ability to inhibit bacterial growth. Computational studies against the major bacterial enzymes of *E. coli* (3HWW), *Salmonella enteritidis* (1D0V), *Staphylococcus aureus* (2RKZ), and *Pseudomonas aeruginosa* (2Q0L) showed the compounds found in *Cordia myxa* can be effective on the targets. The presence of key interactions, such as the possibility of hydrogen bonding and the value of bond energies, inspired the possibility of stable and inhibitory effects on the activity of bacteria. Additionally, the computational forecasts reveal that the hydroxybenzoic acid moiety of *Cordia myxa* can be used as a possible binding site, which additionally underlines its ability to serve as a natural scaffold of bioactive compounds that preserve meat and prevent microbial spoilage, which is a relatively unexplored domain of modern research. The purpose of the study is to identify the bioactive compounds of *C. myxa* and determine its antimicrobial activity. The analysis of the chemical composition showed that it contained phenolic compounds, flavonoids, sterols, saponins, and other biologically active elements (Hamdia et al. 2017).

MATERIALS AND METHODS

Sample preparation procedures

Fresh fruits of *Cordia myxa* were collected from Salah Al-Din Province, Iraq. The fruits were identified by a specialist in the Department of Biology, College of Science, University of Tikrit, Iraq. The fruits were obtained from a local agricultural field under natural environmental conditions. The ripe fruits were carefully cleaned and washed several times with water, followed by rinsing with sterile distilled water. The seeds were then removed, and the pulp was dried under sunlight for two weeks. After

drying, the pulp was ground into a fine powder using an electric mill and then sifted through a 0.5 mm pore mesh. The resulting powder was stored in clean, sterilized brown bottles at room temperature until used in the experiments.

Preparation of ethanolic extract

The ethanolic extract was prepared using a Soxhlet extraction method. Briefly, 50 g of powdered *Cordia myxa* fruit were placed in an extraction thimble and loaded into a Soxhlet apparatus. Subsequently, 500 mL of 80% (v/v) ethanol was added as the extraction solvent, and the process was carried out for 8 h under continuous reflux. Then extract was subsequently concentrated using a rotary evaporator at 45 °C to remove the solvent. Residual alcoholic solvents were further eliminated by placing the extract in an oven at 37 °C for 24 h to obtain a dry powder. After complete drying, the extract was scraped off and stored in airtight containers until further use (Abubakar and Haque 2020). The final weight of the dried extract was 6 g, corresponding to an extraction yield of 12%, which was calculated according to Equation (1).

$$Extraction\ yield(\%) = \frac{Weight\ of\ dried\ extract}{Inicial\ plant\ powder\ weight} \times 100 \qquad (1)$$

A stock solution was prepared at a final concentration of 100 mg mL^{-1}. For reconstitution, the dried extract was dissolved in sterile distilled water under continuous stirring using a magnetic stirrer until complete dissolution. The resulting solution was stored at 4 °C until use.

Alkaloid screening

Alkaloids were detected using Mayer's test, which involved taking 100 mg of extract, adding a few drops of Mayer's reagent, and watching for the development of a yellow precipitate (Arya et al. 2012). The analysis was qualitative in nature, and no quantitative estimation of alkaloid content was performed. Distilled water was used as a negative control, while a standard alkaloid solution was used as a positive control to validate the test results.

Flavonoid screening

Flavonoids were screened using the alkaline reagent and Shinoda tests. In the Shinoda test, a small amount of magnesium were added to 100 mg of the fruit extract, followed by the addition of 1 mL of concentrated hydrochloric acid. The development of pink coloration indicated the presence of flavonoids (Geetha and Geetha 2014). In the alkaline reagent test, 10 mg of the extract was mixed with 1 mL of 10% sodium hydroxide solution, resulting in an intensified yellow coloration. The subsequent addition of 1 mL of concentrated hydrochloric acid caused the solution to become colorless, confirming the presence of flavonoids.

Saponin screening

Based on the Foam test, 100 mg of fruit extract diluted with 20 mL with distilled water and then the mixture completely stirred using a cylinder to form foam up to 1 cm long, which signifies the presence of saponins (Geetha and Geetha 2014).

Phenolic compounds screening

In accordance with the lead acetate test, phenolic chemicals were detected by the development of precipitate when 100 mg of the fruit extract and 5 mL of a 1% lead acetate solution were added (Laouini and Ouahrani 2017).

Tannin screening

The presence of tannins in this extract is indicated by appearance of dark bluish-black color when 5 mL of 5% ferric chloride is added to 100 mg milligrams of the fruit extract according to the ferric chloride test. (Kujur et al. 2010).

Steroid screening

It was performed according to Salkowski's test; 10 mL of concentrated sulfuric acid were added along the walls of the test tube that had 100 mg of fruit extract that had been dissolved in 10 mL of chloroform. The presence of the steroid component in the fruit extract is shown by the lower layer turning yellow with green fluorescence and the top layer turning red (Mukunthan and Balaji 2012).

Meat sample

Five fresh beef samples (thigh muscle) were purchased from a local butcher in Giza, Egypt, on the same day and from the same source to ensure sample uniformity. Each sample weighed 200 g and was cut into uniform slices of 1 cm thickness, then tightly wrapped. The samples were put in sterilized plastic bags and put in a cooling box and transported immediately to the laboratory of the ministry of science and technology where they were to be analyzed.

The beef samples were split into three groups and a control group (200 g per group). The experimental groups were treated with *Cordia myxa* extract in 1, 3, and 5% using the immersion method and left the control group untreated. The extract solution was added to the slices and left to absorb the bioactive compounds and evenly distributed all over the slice in 30 minutes. The samples were then emptied to remove any excess extract and placed in sterile polyethylene bags. Three beef slices in each treatment group were used as independent samples (n=3). All samples were stored under refrigeration at 4 °C for 8 days, following the procedure described by Istrati et al. (2011). Microbiological and chemical analyses were conducted at predetermined storage intervals.

Tested microorganisms

The study involved three Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, and *Pseudomonas aeruginosa* ATCC 10662, as well as one Gram-positive bacterium, *Staphylococcus aureus* ATCC 8095. These strains were provided by the Mycological Center of Assiut University (AUMC) in Assiut, Egypt.

Estimation of the inhibitory effectiveness of *Cordia myxa* extract against tested microorganisms

To evaluate the inhibitory efficacy of the *Cordia myxa* extract against the bacterial isolates, the well diffusion method was employed. Initially, 10 mg of the extract was dissolved into 100 mL of sterile distilled water to prepare a stock solution. This stock solution was then used to prepare the different test dilutions at concentrations of 50, 100, and 150%.

Microbial suspensions were spread evenly on Nutrient Agar plates using sterile swabs. After the agar surface had dried, 5 mm wells were created using a sterilized corn borer. Each well was filled with 0.1 mL of the prepared extract concentrations, while a well-received sterile distilled water. The plates were incubated at 37 °C for 24 hours, and the inhibition zones around the wells were measured in millimeters using a precise ruler to assess antibacterial activity (Jasiem et al. 2016). as shown in Figure 1 Effect of *Cordia myxa* extract on the growth of bacteria.

Activation of bacterial isolates

Microbial strains of *Escherichia coli, Pseudomonas aeroginosa, Salmonella enteritidis*, and *Staphylococcus aureus* were activated using appropriate culture media. The strains were incubated for approximately 18-24 h prior to analysis at 37%. *E. coli* was cultured on MackConkey agar, *P. aeruginosa* on Cetrimide agar, *S. enteritidis* on Hektoen enteric agar, and *S. aureus* on Mannitol salt agar.

Bacterial present in the meat samples were enumerated using the standard plate count method. Visible colonies were counted after incubation, and bacterial populations were expressed as colony-forming units per gram (CFU g^{-1}).

In silico studies

Molecular Docking

Proteins were selected based on their crystallographic resolution and the presence of co-crystallized ligands to ensure reliability for docking analysis. The following Protein Data Bank (PDB) entries were used: 3HWW (Priyadarshi et al. 2009) for *Escherichia coli*, 1D0V (Cheong et al. 2002) for *Salmonella Enteritidis*, 2RKZ (Jida et al. 2023) for *Staphylococcus aureus*, and 2Q0I for *Pseudomonas aeruginosa*. The corresponding three-dimensional co-crystallized structures were retrieved from the Protein Data Bank (https://www.rcsb.org/).

The molecular docking was performed using Molecular Operating Environment (MOE) software. Protein preparation included the removal of water molecules to avoid interference with ligand binding and to improve computational accuracy. Hydrogen atoms were added according to standard geometry, followed by correction of bond connectivity and optimization of the system. The active sites were identified using the Alpha Site Finder tool. For large binding pockets, dummy atoms were generated based on alpha spheres to define the binding region. Ligand-protein interactions with amino acid residues at the active site were subsequently analyzed.

Docking was carried out using the Triangle Matcher placement method and the London dG scoring function. The London dG scoring function was selected because it estimates the free energy of ligand-receptor binding and provides an effective ranking of ligand-protein interactions. The Triangle Matcher method was employed to efficiently generate multiple plausible ligand conformations through geometric matching, ensuring broad exploration of the conformational space. Docking scores were recorded for

all ligand-protein complexes, and the most negative values were considered indicated of the strongest binding affinity. The docking results were considered comparation with the crystallographic conformations of the native ligands. Finally, both two-dimensional and three-dimensional ligand-protein interactions were visualized, as shown in Figure 2.

In silico ADME studies

The Swiss ADME (Daina et al. 2017) was used to analyze the physicochemical pharmacokinetic properties, drug-like nature, and suitability for medicinal chemistry parameters of *p*-coumaric acid.

Analysis of *Cordia myxa* by HPLC Chromatogram

The ethanolic extract from *Cordia myxa* fruit was analyzed using HPLC to identify phenolic compounds. A C18 column (250 × 4.6 mm, 5 µm particle size) was used for separation. The mobile phase consisted of a mixture of methanol and water containing 0.1% formic acid, and the system was operated under gradient elution conditions to achieve optimal resolution of the compounds. Detection was performed at a wavelength of 280 nm, and the retention times for each identified phenolic compound, gallic acid, ferulic acid, caffeic acid, p-coumaric acid, and chlorogenic acid. Quantification of each compound was conducted using standard calibration curves prepared from known concentrations of reference standards. Each analysis was performed in duplicate to ensure accuracy and reliability of the result (Skoog et al. 2013)

Statistical analysis

The Statistical Packages of Social Sciences (SPSS 2019) program was used to detect the effect of different groups/ factors in study parameters. Least significant difference-LSD was used to significant compare between means in this study.

RESULTS AND DISCUSSIONS

Active compounds in *Cordia myxa* extract

The phytochemical screening Table 1 demonstrated that the alcoholic extract of *Cordia myxa* is rich in bioactive, including alkaloids, tannins, phenolic compounds, flavonoids, saponins, and steroids. The extraction of these constituents underscores the efficiency of ethanol as an extraction solvent especially in extracting phenolic compounds like phenolics and flavonoids which are said to be rich in *Cordia myxa* fruits (Abdel-Aleem et al. 2019). It is especially important to emphasize the identification of phenolic compounds and flavonoids because these classes of metabolites have been relatively well-known to possess redox properties, which allows them to scavenge free radicals and reduce oxidative stress. In addition to antioxidant action, phenolics have antimicrobial effects by a variety of the following mechanisms; enzyme inhibitory, metal ion chelation, and destabilization of microbial membrane integrity. These results are consistent with those of Hamdia et al. (2017), who found high levels of phenolics and significant biological processes in *Cordia myxa* extracts.

Table 1. Results phytochemical testes of alcoholic *Cordia myxa* extract.

No.	Phytochemical Testes	Results
1	Alkaloids	+
2	Tannins	+
3	Phenolic compounds	+
4	Flavonoids	+
5	Saponins	+
6	Steroids	+

*Screening test in triplicate.

Tannins help in antimicrobial action by coagulation of proteins and the formation of complexes with the cell wall, thus weakening bacterial multiplication. Alkaloids have the ability to disrupt the synthesis of nucleic acid and key metabolic pathways, whereas saponins are reported to affect the membrane permeability through interaction with membrane sterols, leading to cellular leakage. Steroids can also increase antimicrobial activity through the effects of membrane destabilization.

The coexistence of all these various classes of phytochemicals is an indication that there might be a synergistic interaction, with a combination of bioactive compounds acting in a complementary manner. This biological diversity can be the reason behind the biological effectiveness of the extract and support its potential as a natural food preservative and functional food ingredient in food systems and can be considered an effective alternative to synthetic food additives.

Inhibitory activity of alcoholic extract on several bacterial isolates

Table 2 shows the antimicrobial effect of the alcoholic

extract of dried *Cordia myxa* on the selected bacterial isolates in concentration of 50, 100 and 150%. The statistical analysis proved that there were significant differences ($P \leq 0.05$) between the tested concentrations in growth inhibition which was a concentration-dependent response.

It was found that the inhibitory activity increased gradually with the concentration of the extracts. Inhibition zone diameter was 11.0, 13.0, and 17.6 mm at 50, 100 and 150 in the case of *Escherichia coli*. On the same note, *Salmonella enteritidis* had an inhibition zone of 9.4, 11.2 and 15.0 mm under the same concentration gradient.

Table 2. Inhibition zone (mm) *Cordia myxa* extract.

Bacteria	50 (mg L^{-1})	100 (mg L^{-1})	150 (mg L^{-1})	C	P-value
E. coli	11.0±0.52	13.0±0.72	17.6±0.87	0±0	0.0001**
Salmonella enteritidis	9.4±0.46	11.2±0.67	15.0±0.43	0±0	0.0001**
Staphylococcus aureus	12.0±0.61	16.0±0.86	18.5±0.92	0±0	0.0001**
Pseudomonas aeruginosa	12.1±0.65	13.2±0.70	14.0±0.75	0±0	0.0001**
P-value	0.0375*	0.0082**	0.0237*	NS	---
		* ($P \leq 0.05$), ** ($P \leq 0.01$).			

*Significant ($P \leq 0.05$). Highly Significant ($P \leq 0.01$). Not Significant (NS). *The values represent the mean of three replicates.

In Gram-positive *Staphylococcus aureus*, the extract showed greater susceptibility and the diameters of the inhibition zones grew with 12.0 mm at 50% to 18.5 mm at 150%. Similar concentration-related trend was observed in the *Pseudomonas aeruginosa*, which exhibited the inhibition diameters of 12.1, 13.2, and 14.0 mm, respectively.

All these findings present strong evidence on the dose-responsive antibacterial effect of the extract against Gram-negative and Gram-positive bacteria. The efficacy that is observed can be explained by the existence of bioactive phenolic and flavonoid compounds which can disrupt the integrity of microbial cell membranes, enhance membrane permeability, and cause oxidative stress in bacterial cells. These chemicals can also disrupt normal metabolic processes and the production of nucleic acids. Also, tannins play their role in the form of protein precipitation, and saponins are involved in increasing membrane destabilization, which indicates that the antibacterial effect is probably a synergistic effect of various active constituents. The findings support the previous findings (Al-Musawi et al. 2022), which also reported high antibacterial effects of *Cordia myxa* extracts.

Microbial tests of meat treatments during different storage periods

Table 3 shows the microbial counts in beef samples stored for 8 days at 4 °C, with and without the addition of *C. myxa* alcoholic extract. The results of the statistical analysis indicate that there are significant differences at the significance level ($P<0.05$) significant reduction in various types of microorganisms in the treated samples compared to the control. This supports the effectiveness of alcoholic extracts in preserving meat by inhibiting microbial proliferation. Although microbial growth increased over time in all samples, the presence of the extract significantly limited this increase. The microbial loads in treated groups remained consistently lower than in the control group throughout the storage period, confirming the preservative potential of *Cordia myxa* (Hamdia et al. 2017).

Escherichia coli counts of the treated groups B1, B2 and B3 comprised of 2.65, 2.42 and 2.18 log CFU g^{-1}, respectively, on the first day of storage as compared to 2.81 of the control. On the third day the bacterial growth was observed to be 3.17 (B1), 3.05 (B2), and 2.36 (B3) with the control being 3.22. On the fifth day, *E. coli* levels increased to 4.23, 3.64, and 3.24 in B1, B2, and B3, respectively, with control at 5.30. By the eighth day, the counts rose further to 6.59 (B1), 5.19 (B2), and 4.74 (B3), whereas the control group reached 8.82 log CFU g^{-1}. On the first day of storage, *S. entertites* counts were 3.10 (B1), 2.58 (B2), and 2.00 (B3) log CFU g^{-1}, compared to 3.81 in the control. By day three, the levels increased to 4.55 (B1), 3.23 (B2), and 2.26 (B3), while the control reached 5.22. On Day five, there was

a high of 5.36 (B1), 3.94 (B2), and 3.17 (B3) and the control stood at 5.58. On the eighth day, the result was 6.92 (B1), 5.47 (B2) and 5.02 (B3) compared to 7.07 in the control. In the case of *Staphylococcus aureus*, B1 (1.86), B2 (1.52), and B3 (1.13) were counted on the first day, and the count was 2.11 in the control. On day three, the value rose to 2.68 (B1), 2.18 (B2), and 1.65 (B3), while the control was 3.29. By day five, growth reached 3.71 (B1), 3.25 (B2), and 2.59 (B3), versus 4.08 in control. On day eight, *S. aureus* levels were 5.21 (B3), 5.01 (B2), and 3.75 (B1), compared to 6.52 in the control group. These results align with findings confirming the inhibitory effect of *C. myxa* extract on microbial growth (Jooyandeh et al. 2023). On the first day of storage, the numbers of growth of *Pseudomonas aeruginosa* bacteria reached in the different treatments B1 (3.0), B2 (2.68), and B3 (1.67), compared to the control treatment, which reached (3.4) Cell. On the third day of storage, the numbers of growth of bacteria they reached in the different treatments (3.85, 3.22, and 2.54) compared to the control treatment, which reached (4.2) Cell.

On the fifth day of storage, the growth of the number of *P. aerogonsa* in the different treatments B1, B2, and B3 reached 4.37, 3.89, and 3.77, respectively, compared to the control treatment, which reached 5.1 cells. The growth of the number of *P. aerogonsa* on the eighth day of storage in the treatments B3, B2, and B1 reached 6.22, 6.01, and 5.32, respectively, compared with the control treatment which amounted to 7.80.

Table 3. Effects of *Cordia myxa* on microbiological in colony-forming unit (CFU g^{-1}) of beef stored at 4 °C for 8 days.

Types of bacteria	Sample type	Storage days				P-value
		1	3	5	8	
E. coli	**C**	2.81±0.13	3.22±0.08	5.30±0.24	8.82±0.57	0.0001**
	B1	2.65±0.10	3.17±0.19	4.23±0.21	6.59±0.42	0.0044**
	B2	2.42±0.07	3.05±0.14	3.64±0.15	5.19±0.38	0.0276*
	B3	2.18±0.07	2.36±0.08	3.24 ±0.18	4.74±0.25	0.0417*
Salmonella Enteritidis	**C**	3.81±0.16	5.22±0.31	5.58±0.36	7.07±0.58	0.0092**
	B1	3.10±0.12	4.55±0.26	5.36±0.32	6.92±0.7	0.0385*
	B2	2.58±0.08	3.23±0.20	3.94±0.19	5.47±0.38	0.0289*
	B3	2.0±0.06	2.26±0.11	3.17±0.1	5.02±0.34	0.0251*
Staphylococcus aureus	**C**	2.11±0.07	3.29±0.18	4.08 ±0.23	6.52±0.42	0.0039**
	B1	1.86±0.05	2.68±0.09	3.71±0.20	5.21±0.35	0.0088**
	B2	1.52±0.05	2.18±0.07	3.25±0.17	5.01±0.31	0.0082**
	B3	1.13±0.03	1.65±0.05	2.59±0.14	3.75±0.20	0.0392*
Pseudomonas aeruginosa	**C**	3.40 ±0.24	4.2±0.28	5.10±0.34	7.80±0.46	0.0026**
	B1	3.0±0.18	3.85±0.21	4.37±0.26	6.22±0.41	0.0281*
	B2	2.68±0.23	3.22±0.12	3.89±0.25	6.01±0.44	0.0063**
	B3	1.67±0.08	2.54±0.17	3.77±0.21	5.32±0.32	0.0048**
		0.0001**	0.0001**	0.0001**	0.0001**	--
				*($P \leq 0.05$), **($P \leq 0.01$).		

C=Control, B1= 1% of the extract, B2= 3% of the extract, B3= 5% of the extract. *Significant ($P \leq 0.05$). Highly Significant ($P \leq 0.01$). Not Significant (NS). *The values represent the meaning of three replicates.

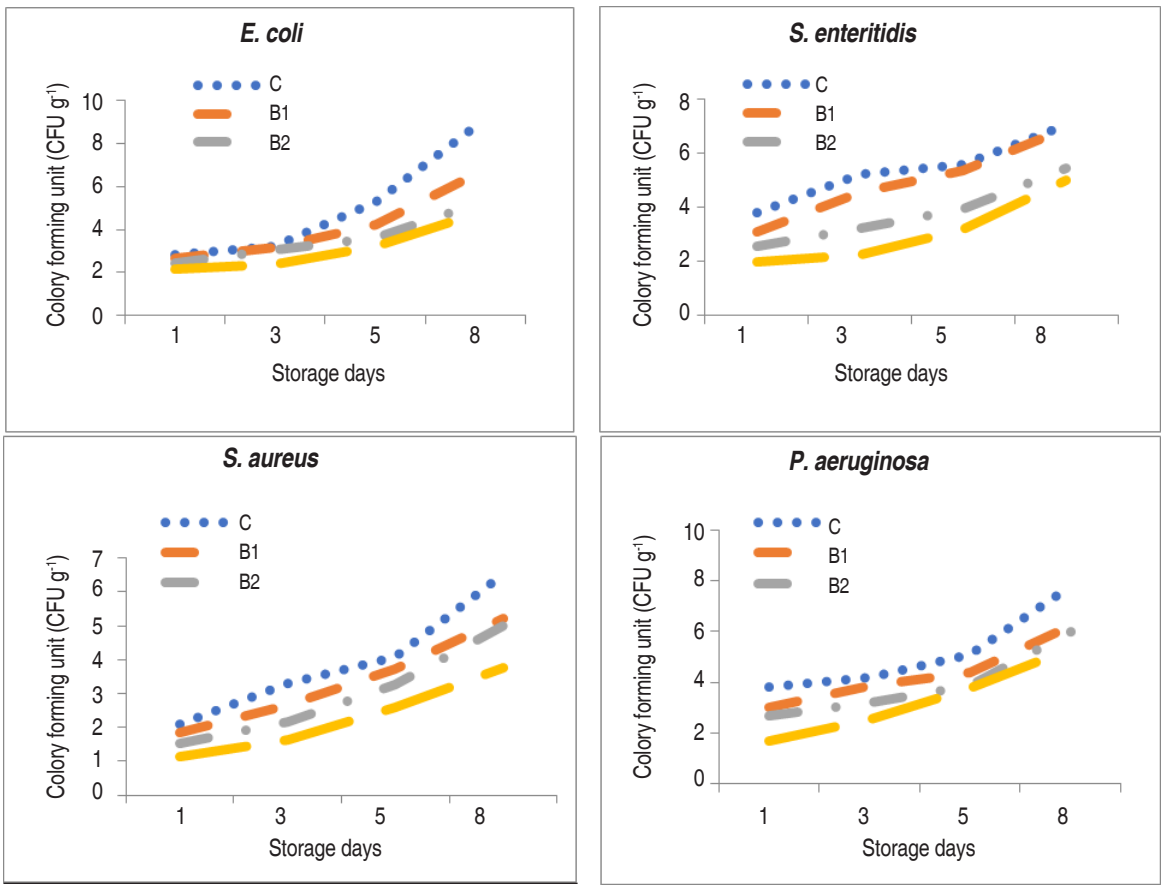


Figure 1. Effect of *Cordia myxa* alcoholic extract on the growth of beef spoilage bacteria during storage at 4 °C for 8 days.

The most significant reduction in microbial load was observed in beef samples treated with 5% *C. myxa* extract, which outperformed the 3 and 1% concentrations. The antimicrobial effect was also observed to last during the 8 days refrigeration period and this means that it was still inhibitory. Although meat is mixed with microorganisms that can occur naturally, its growth is affected by conditions conducive to its growth including moisture, temperature and oxygen. The contamination may also be done during slaughtering, cutting, processing and poor hygiene or storage facilities. The decrease in the contents of microbes can be explained by the fact that *Cordia myxa* has flavonoid and phenolic compounds that have been identified to have antimicrobial effects. By binding to cell wall proteins and weakening the integrity of the membranes, these compounds interfere with the functioning of microbial cells (Al-Musawi et al. 2022). The research affirms that active components of the extract including alkaloids, flavonoids, phenols, and tannins, all of which are active components, are rendered highly effective regarding its antibacterial activity. These results are in accordance with the past studies proving the potential of *Cordia myxa* as a natural food preservative.

Validation of molecular docking protocol

One of the most widely used methods for validating a docking protocol is the re-docking of co-crystallized ligand to the target protein. In this study, the co-crystallized ligand was extracted from the receptor protein and re-docked into the active site of the respective receptor. After docking, the best pose of the ligands was aligned with the co-crystallized ligand and the RMSD of the ligand was calculated. This helps to determine the reliability and reproducibility of the docking protocol. All docked conformations of ligands when superimposed

with respective co-crystallized ligands were within an RMSD of 2.0 A˚, indicating a valid docking protocol. The receptor-inhibitor pairs were 3HWW with AKG, 1D0V with NIO, 2RKZ with SIN, and 2Q0I with BEZ in analyzing docking scores and interactions formed (Figure 2 and Table 4).

Analysis of molecular docking study

The protein was selected accordingly:

- Target potential biological strategies as 3HWW **(**Priyadarshi et al. 2009**)** for *E. coli*, PDB ID: 1D0V (Cheong et al. 2002) for *Salmonella*, PDB ID: 2RKZ (Jida et al. 2023) for *Staphylococcus aureus,* and PDB ID: 2Q0I (Yu et al. 2009) for *Pseudomonas aeruginosa.*
- All of their resolutions are acceptable with the documented protocol [≤ 2].
- All co-crystallized ligands (CCL), which are included in the target proteins, contain alcohol using X-ray as reported in each target protein. Alcohol is an antimicrobial agent.
- The binding energy of ligands was found to be higher than that of the CCL, implying that they fit with high stability.
- The low value of RMSD, i.e., the distances between the active site protein and ligands, is the closeness of the functional groups involved.
- The interactions seemed to include potential kinds, including H-donor, H-acceptor, and π-H.
- The best protein-ligand interaction for these tested ligands is Ser411, Glu233, Gly113, Ser285, Asp73, Gly202, Gly264, Phe265, Ser291, Met177, and Gly176.
- The O and phenyl moiety's sites were the key objectives of the ligation mode.

Table 4. Energy scores (kcal moL^{-1}), RMSD (A˚) H-bonds formed between the test ligand with the target antimicrobial receptors when compared to the native co-crystallized ligand.

PDB ID	Compound	Energy score (S) (kcal moL^{-1})	RMSD (A˚)	H-bonds
3HWW	Gallic a`	-5.17	0.81	H-bond donor with Ser411 H-bond acceptor with Ser411
	Caffeic a`	-5.28	0.75	-
	p-Coumaric a`	-5.30	1.31	H-bond donor with Ser411 H-bond acceptor with Glu233
	CCL	-4.81	1.91	H-bond donor with Ser411
1D0V	Gallic a`	-4.82	0.63	H-bond donor with Gly202 H-bond acceptor with Gly264 H-bond acceptor with Phe265 H-bond acceptor with Ser291
	Caffeic a`	-5.10	0.90	-
	p-Coumaric a`	-5.16	1.83	H-bond donor with Met177 H-bond acceptor with Gly176
	CCL	-4.51	0.61	H-bond acceptor with Met177 H-bond acceptor with Gly176
2RKZ	Gallic a`	-3.79	1.10	-
	Caffeic a`	-3.82	1.16	-
	p-Coumaric a`	-3.87	1.35	-
	CCL	-3.60	1.10	-
2Q0I	Gallic a`	-4.33	0.59	H-bond donor with Ser285
	Caffeic a`	-4.67	0.53	-
	p-Coumaric a`	-5.51	0.79	H-bond donor with Asp73
	CCL	-4.09	0.94	H-bond donor with Asp73

A crystallized inhibitor in combination with the receptor was used to perform the redocking process. The RCSB PDB has receptors with bound crystallized inhibitors. The receptor-inhibitor pairs were 3HWW with AKG, 1D0V with NIO, 2RKZ with SIN, and 2Q0I with BEZ in analyzing docking scores and interactions formed (Figure 2 and Table 4). Molecular docking serves as a valuable tool for predicting potential drug candidates by evaluating how well compounds interact with specific protein targets. The results of docking have been graphically illustrated in Figure 2 and demonstrate significant interactions used in antimicrobial inhibition. In this study, p-coumaric acid has been chosen as the test compound and the receptors were 3HWW, 1D0V, 2RKZ and 2Q0I. Generally, compounds with more negative binding energy values indicate stronger and more favorable interactions with the target proteins.

Molecular docking began with the *E. coli* receptor 3HWW and its co-crystallized ligand (AKG), which showed a binding energy of -4.81 kcal moL^{-1}, higher (i.e., weaker) than the test ligand. Among all tested compounds, p-coumaric acid exhibited the strongest binding to 3HWW, with a binding energy of -5.30 kcal moL^{-1}, forming two hydrogen bonds via its hydroxyl group.

In a second study, 1D0V was used as the receptor for *Salmonella enteritidis*, paired with NIO as the co-crystallized ligand, which had a binding energy of 4.51 kcal moL^{-1}. p-Coumaric acid again showed stronger binding, with -5.16 kcal moL^{-1}, forming hydrogen bonds with Met177 (donor) and Gly176 (acceptor).

The third docking involved the *Staphylococcus aureus* receptor 2RKZ and SIN as its reference ligand, which had a binding energy of -3.60 kcal moL^{-1}. p-Coumaric acid showed improved affinity with -3.87 kcal moL^{-1}, interacting through a pi-H bond between its phenyl ring and the Gly113 residue of the receptor. The final docking was performed with the 2Q0L *Pseudomonas aeruginosa* receptor, using BEZ as a CCL. It showed a binding energy of -4.09 kcaL moL^{-1}. Test ligand *p*-coumaric acid had binding energy of -5.51 kcal moL^{-1}, when they interacted with their target enzyme. Interactions between test ligand and 2Q0L that formed hydrogen bonding donor of *p*-coumaric acid. It has been established that the existence of hydrogen bonding is one of the most essential variables in enhancing the binding affinity of ligands and protein receptors. The selectivity of ligand binding and its importance for the characteristics of biomacromolecules are determined by hydrogen bonding (Nallal et al. 2021). The most important characteristic influencing the higher binding affinity between medication candidates and receptors is strong hydrogen bonds (López-Yerena et al. 2020). The interactions may improve the drug target's interface's binding affinity.

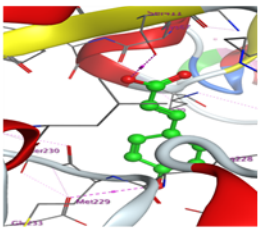
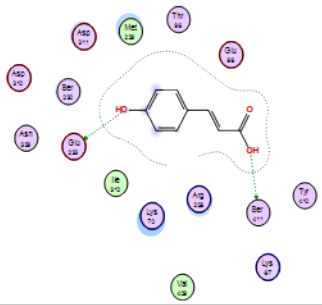
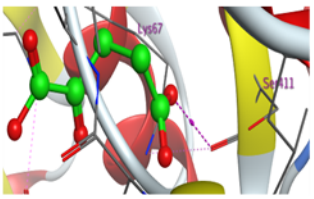
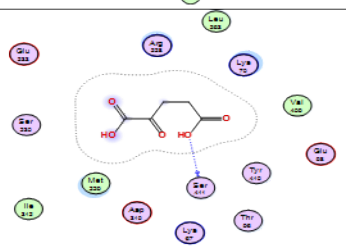
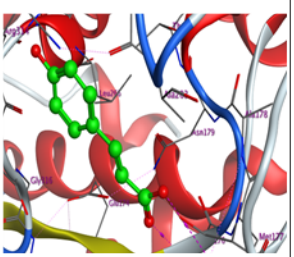
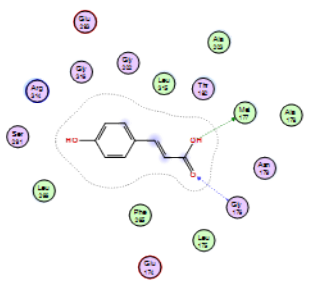
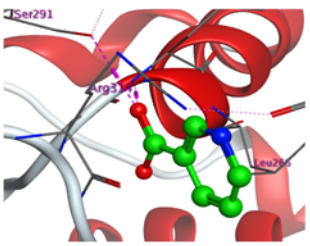
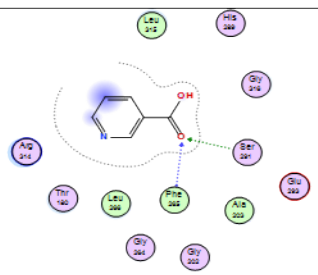
This research revealed that: (I) CCL compounds exhibited lower binding energies compared to the test ligand; (II) among the test ligands, *p*-coumaric acid demonstrated the highest binding energy with all target receptors. These results suggest that *p*-coumaric acid is the most promising candidate, and the tested ligand may serve as potential antimicrobial agents.

These compounds are known for their diverse biological and pharmacological activities. Gallic acid exhibits antimicrobial, anti-inflammatory, antioxidant, and antineoplastic properties. Ferulic acid is recognized for its antioxidant and pharmacological effects. Chlorogenic acid is known for its antidiabetic, DNA-protective, and therapeutic roles. Caffeic acid shows anti-cancer, anti-toxicity, anti-diabetic, and anti-inflammatory properties. *P*-coumaric acid is noted for its antioxidant, antimicrobial, antitumor, and anti-inflammatory activities (Al-Musawi et al. 2022).

The molecular docking study utilized high-resolution crystal structures obtained from the Protein Data Bank. The *E. coli* target, MenD, was represented by PDB ID 3HWW, complexed with oxoglutarate (CCL). Bacterial respiration and electron transport depend on menaquinone. By interfering with this system of operation, inhibition of MenD affects bacterial metabolism of energy.

PDB ID 1D0V corresponds to CobT from *Salmonella typhimurium*, resolved at 1.9 A° and co-crystallized with its reaction products. Cobalamin production is disrupted when CobT is inhibited, which hinders bacterial growth and survival. CobT is regarded as an antibacterial target that is selective (Claas et al. 2010).

For *Staphylococcus aureus*, PDB ID 2RKZ was selected, depicting fibronectin-binding protein A (FnBPA-1) in complex with fibronectin modules. Fibronectin functions as a bridge between bacterium and host cell. It promotes

Receptors	Cpd.	3D Ligand interaction	2D Ligand interaction
3HWW	*p*-Coumaric a`		
	CCL		
1D0V	*p*-Coumaric a`		
	CCL		

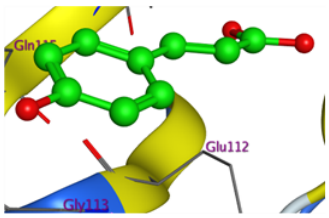
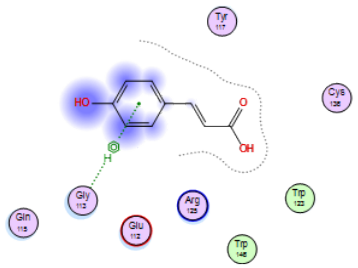
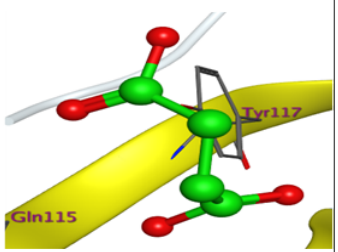
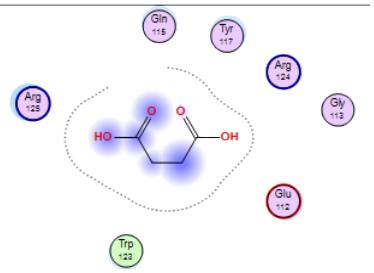
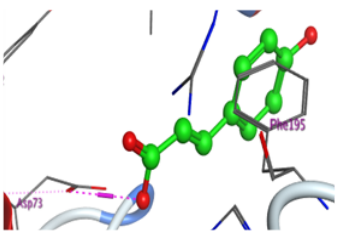
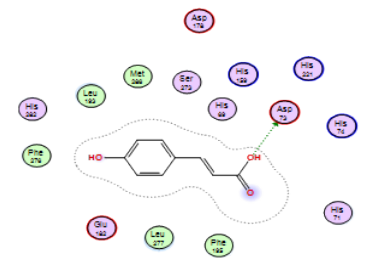
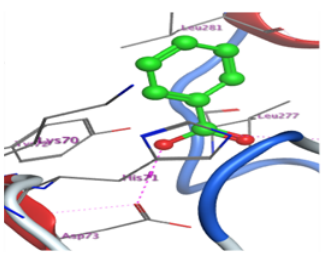
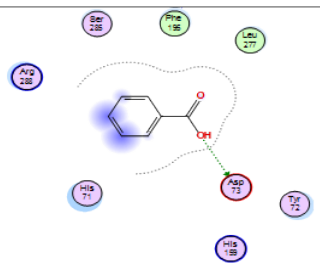
2RKZ	*p*-Coumaric a`		
	CCL		
2Q0I	*p*-Coumaric a`		
	CCL		

Figure 2. 3D and 2D interactions of p-coumaric acids and CCL with antimicrobial receptors (3HWW, 1D0V, 2RKZ, 2Q0I).

adherence to and aggregation of activated platelets independently of other *S. aureus* surface molecules. It is promoting both colonization and persistence of the bacterium into the host.

Finally, PDB ID 2Q0I represents *Pseudomonas* species, thioredoxin reductase from *Helicobacter pylori*, reduced by sodium dithionite and complexed with $NADP^+$. It is a component of the 2-aminophenol (2AP) catabolic pathway

across a number of bacteria, including *Pseudomonas* species. Bacteria can use aromatic amines as sources of nitrogen related to the 2AP pathway, which promotes development in conditions with limited nutrients. Enzymes are possible selective targets for antibacterial because this pathway is specific to bacteria and is not found in humans. Inhibiting them could prevent bacteria from using aromatic chemicals, which would reduce their ability to survive. These protein models served as representative bacterial targets for evaluating ligand-binding affinities.

Design and evaluation of physicochemical, drug likeness, and medicinal chemistry prediction properties

Lower molecular weight and lipophilicity molecules are expected to have improved absorption properties as well as a transporter impact on clearance, resulting in improved renal excretion and moderate toxicity (Struck et al. 2008). The representation of physicochemical features data indicates that the molecular weights of the suggested compound fall in 164.16 g moL^{-1} as illustrated in Table 5 of computed values for *p*-coumaric acid. A molecule is considered to be a drug-like molecule (DLM) when it satisfies the requirements outlined in the "rule of five" (Ro5). When a molecule's molecular mass is less than 500, its log P is less than 5, and its hydrogen bond donors and acceptors are less than 5 and 10, respectively, it meets the Ro5 criterion. Two hydrogen bond donors (HD) and three hydrogen bond acceptors (HA) were found in the compound under test. The MLOGP value of *P*-coumaric acid is 1.28. According to the Veber rule, compounds have better oral bioavailability if their rotatable bonds, polar surface area, and H-bond donors and acceptors are equal to or less than 10, 140 and 12

Table 5. Predicted Bioactivity and ADME Parameters of *p*-Coumaric Acid.

Category	Parameter	Value
Molecular Properties	Molecular Formula	$C_9H_8O_3$
	Heavy Atoms Count	12
	Rotatable Bonds	2
	Aromatic Atom Count	6
	Fraction of sp^3 Carbons	0.00
	Molar Refractivity	45.13
	Topological Polar Surface Area (TPSA)	57.53 $A^{\circ 2}$
Polarity	Hydrogen Bond Acceptors	3
	Hydrogen Bond Donors	2
Solubility and Lipophilicity	Aqueous Solubility	Soluble
	Log Po/w (MLOGP)	1.28
	Log S (ESOL model)	-2.02
Pharmacokinetics	Gastrointestinal Absorption	High
	Skin Permeability (log Kp)	-6.26 cm s^{-1}
	P-glycoprotein Substrate	No
	Blood-Brain Barrier Penetration	Yes
Drug-Likeness	Bioavailability Score	0.85
	Lipinski's Rule of Five	Compliant (0 violations)
	Veber Filter	Passed
Medicinal Chemistry	Synthetic Accessibility Score (1–10)	1.61
	Lead-like Compound	No
	PAINS Alerts	None

*Swiss ADME (Daina et al. 2017).

(Veber et al. 2002). Compounds have two rotatable bonds. The oral bioavailability of the chemical is low if the TPSA score is greater than 130 $A^{\circ 2}$ while *p*-coumaric acid had the TPSA value of 57.53 $A^{\circ 2}$ with an excellent oral bioavailability profile.

Additionally, the studied derivative demonstrated solubility for water solubility prediction, which, according to the ESOL model, exhibited soluble characteristics, including a log *S* value of no more than 6.

Evaluated hydroxybenzoic acid derivative demonstrated high gastrointestinal absorption (GIA); notably, *p*-coumaric acid also exhibited a blood–brain barrier (BBB) equal to -0.225 log BB and its CNS permeability -2.418 log PS, providing evidence for focused antimicrobial application. None of the compounds were identified as P-glycoprotein (P-gp) substrates. Moderate skin permeability was observed, with Log Kp value is -6.26 cm s^{-1}. It became evident that strong medical findings were obtained, which represents a key strength of the study and contributes to opening an important medical field for future research. Physicochemical analysis showed that compounds complied with Lipinski's and Veber's rules, indicating good drug-likeness. *P*-coumaric acid had the bioavailability score (0.85). There are no PAINS (pan-assay interference compounds) alerting for *p*-coumaric acid. However, derivatives were considered synthetically accessible, with synthetic accessibility scores between 1.61.

Phytochemicals and plant extracts from natural sources are known for their strong biological properties, including anticancer activity, and are often considered more effective or safer alternatives to conventional chemotherapy and traditional medicines (Watkins et al. 2015).

CONCLUSION

The present study demonstrated that the alcoholic extract of *Cordia myxa* fruit significantly improved the microbiological quality of refrigerated beef stored at 4 °C for up to 8 days. Out of the tested concentrations, the 5% extract showed the greatest reduction in microbial load and this shows that it is an effective natural preservative against meat storage. The antimicrobial activity observed could be attributed to the presence of the phenolic components observed in the extract.

Nevertheless, the research was restricted to microbiological analysis under laboratory conditions and was not accompanied by the detailed evaluation of physicochemical values or sensory qualities of the treated meat. Also, storage was limited to eight days, which might not be representative in extended commercial storage.

More studies are suggested in order to assess sensory properties (color, odor, texture, and acceptability) of the extract and determine the effectiveness of the extract as a preservative after longer periods of storage in real application conditions. This is a type of study that needs to be conducted to prove that it can be used as a natural preservative in meat product.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest.

REFERENCES

Abdel-Aleem ER, Attia EZ, Farag FF, Samy MN and Desoukey SY (2019) Total phenolic and flavonoid contents and antioxidant, anti-inflammatory, analgesic, antipyretic and antidiabetic activities of *Cordia myxa* L. leaves. Clinical Phytoscience 5 (29): 1-9. https://doi.org/10.1186/s40816-019-0125-z

Abubakar AR and Haque M (2020) Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences 12 (1). https://doi.org/10.4103/jpbs.JPBS_175_19

Al-Musawi MH, Ibrahim KM and Albukhaty S (2022) *In vitro* study of antioxidant, antibacterial, and cytotoxicity properties of Cordia myxa fruit extract. Iranian Journal of Microbiology 14(1): 97. https://doi.org/10.18502/ijm.v14i1.8810

Al-Musawi MH, Ibrahim KM and Albukhaty S (2022) Phytochemical analysis and antimicrobial activities of ethanol extract of *Cordia myxa* fruit: *In vitro* study. Research Journal of Pharmacy and Technology 15 (7): 2871–2876. https://doi.org/10.52711/0974-360X.2022.00479

Arya V, Thakur N and Kashyap CP (2012) Preliminary phytochemical analysis of the extracts of *Psidium* leaves. Journal of Pharmacognosy and Phytochemistry 1(1): 1–5. https://www.phytojournal.com/vol1Issue1/Issue_may_2012/1.1.pdf

Cheong CG, Escalante-Semerena JC and Rayment I (2002) Capture of a labile substrate by expulsion of water molecules from the active site of (nicotinate mononucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase) (CobT) from *Salmonella enterica*. Journal of Biological Chemistry 277:43. https://doi.org/10.1074/jbc.M203535200

Claas KR, Parrish J, Maggio-Hall L and Escalante-Semerena J (2010) Functional analysis of the nicotinate mononucleotide: 5, 6-dimethylbenzimidazole phosphoribosyltransferase (CobT) enzyme, involved in the late steps of coenzyme B12 biosynthesis in Salmonella enterica. Journal of bacteriology 192(1): 145-154. https://doi.org/10.1128/jb.01159-09

Daina A, Michielin O and Zoete V (2017) SwissADME: A free

web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. Scientific Reports 7: 42717. https://doi.org/10.1038/srep42717

Geetha TS and Geetha N (2014) Phytochemical screening and quantitative analysis of primary and secondary metabolites of *Cymbopogon citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. International Journal of PharmTech Research 6 (2): 521–529. https://www.researchgate.net/publication/287346735

Hamdia D, Al-Hamdani MS and Al-Faraji DA (2017) Evaluation of inhibitory activity of *Cordia myxa* fruit extract on microorganisms that causes spoilage of food and its role in the treatment of certain disease states. Journal of Biology, Agriculture and Healthcare 7 (2): 2224–3208. https://api.semanticscholar.org/CorpusID:54765657

Hussein HM, Mohsen E, Abdel-Monem AR and Abdel Kawy M (2023) An overview on botanical characteristics, phytochemical constituents and pharmacological effects of *Cordia dichotoma* (G. Forst.) and *Cordia sebestena* (L.) (Boraginaceae). Egyptian Journal of Chemistry 66(13): 437–459.

Istrati D, Constantin O, Ionescu A, Vizireanu C and Dinică R (2011) Study of the combined effect of spices and marination on beef meat vacuum packaged. The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology 35(2): 75–85.

Jasiem TM, Al-Mugdadi SFH, Aljubory IS and Latef QN (2016) Phytochemical study and antibacterial activity of crude alkaloids and mucilage of *Cordia myxa* in Iraq. International Journal of Pharmaceutical Sciences Review and Research 39 (1): 232–236. https://www.researchgate.net/publication/306158913

Jida MD, Rajaselvi DN, Nisha AR, Sujith S, Suja Rani S and Panicker VP (2023) In silico molecular docking study of milk-derived peptide against biofilm-forming *Staphylococcus aureus* and *Escherichia coli*. The Journal of Phytopharmacology 12 (6): 421–428. https://doi.org/10.31254/phyto.2023.12608

Jooyandeh H, Kaykha MEH, Behbahani BA and Noshad M (2023) Evaluating the quality of mutton meat coated with *Cordia myxa* fruit mucilage containing *Rosmarinus officinalis* essential oil during cold storage. Journal of Food Measurement and Characterization 17 (3): 2062–2074. https://doi.org/10.1007/s11694-022-01788-2

Kargiotou C, Katsanidis E, Rhoades J, Kontominas M and Koutsoumanis K (2011) Efficacies of soy sauce and wine-based marinades for controlling spoilage of raw beef. Food Microbiology 28 (1): 158–163. https://doi.org/10.1016/j.fm.2010.09.013

Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S and Roy BK (2010) Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. Pharmacognosy and Natural Products 2 (14): 27–32. https://doi.org/10.4103/0974-8490.69128

Laouini SE and Ouahrani MR (2017) phytochemical screening, *in vitro* antioxidant and antibacterial activity of *Rumex vesicarius* L. extract. Scientific Study and Research: Chemistry and Chemical Engineering 18 (4): 367 – 376. https://doaj.org/article/ea8359e95bd74528b7bca1e183ef7ead

López-Yerena A, Perez M, Vallverdú-Queralt A and Escribano-Ferrer E (2020) Insights into the binding of dietary phenolic compounds to human serum albumin and food-drug interactions. Pharmaceutics 12(11): 1123. https://doi.org/10.3390/pharmaceutics12111123

Mukunthan KS and Balaji S (2012) Cashew apple juice (*Anacardium occidentale* L.) accelerates the synthesis of silver nanoparticles. International Journal of Green Nanotechnology 4 (2): 71–79. https://doi.org/10.1080/19430892.2012.676900

Nallal VUM, Padmini R, Ravindran B, Chang SW et al (2021) Combined *in vitro* and in silico approach to evaluate the inhibitory potential of an underutilized *Allium* vegetable and its bioactive compounds on multidrug-resistant *Candida* species. Saudi Journal of Biological Sciences 28 (2): 1246–1256. https://doi.org/10.1016/j.sjbs.2020.11.082

Pathania A, McKee SR, Bilgili SF and Singh M (2010) Antimicrobial activity of commercial marinades against multiple strains of *Salmonella* spp. International Journal of Food Microbiology 139 (3): 214–217. https://doi.org/10.1016/j.ijfoodmicro.2010.01.039

Priyadarshi A, Kim EE and Hwang KY (2009) Structural and functional analysis of Vitamin K2 synthesis protein MenD. Biochemical and Biophysical Research Communications 388 (4): 748–751. https://doi.org/10.1016/j.bbrc.2009.08.093

Skoog DA, West DM, Holler FJ and Crouch SR (2013) Fundamentals of Analytical Chemistry (9th ed.). Cengage Learning.

SPSS - Statistical Packages of Social Sciences (2019) Statistical Packages of Social Sciences-SPSS/ IBM Statistics 26 step by step. 16th Edition. https://doi.org/10.4324/9780429056765

Struck S, Schmidt U, Gruening B, Jaeger IS, Hossbach J and Preissner R (2008) Toxicity versus potency: Elucidation of toxicity properties discriminating between toxins, drugs, and natural compounds. Genome Informatics 20: 231–242. https://pubmed.ncbi.nlm.nih.gov/19425137/

Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW and Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry 45: 2615–2623. https://doi.org/10.1021/jm020017n

Wani AY, Khaliq R, Kousar N and Pundir S (2024) The pharmacognostic profile and pharmacological importance of *Cordia myxa*: A review. Computational Algorithms and Numerical Dimensions 2 (3): 186. https://doi.org/10.22105/cand.2024.437572.1086

Watkins R, Wu L, Zhang C, Davis RM and Xu B (2015) Natural product-based nanomedicine: Recent advances and issues. International Journal of Nanomedicine 10: 6055–6063. https://doi.org/10.2147/ijn.s92162

Yu S, Jensen V, Seeliger J, Feldmann I, Weber S, Schleicher E et al (2009) Structure elucidation and preliminary assessment of hydrolase activity of PqsE, the Pseudomonas quinolone signal (PQS) response protein. Biochemistry 48(43): 10298-10307.