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Phytochemical analysis and in vitro and in vivo pharmacological activities of *Cordia myxa* extracts

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Abstract: *Cordia myxa* is found in tropical regions of Pakistan, where it is commonly known as lasura. This study aimed to assess the preliminary results of phytochemical analysis and the pharmacological activities of *C. myxa*. After pulverizing the fresh plant, extracts were prepared using different solvents, including aqueous, methanol, ethanol, dichloromethane, ethyl acetate, and *n*-hexane extracts. These extracts were examined for total phenolic contents using standard biochemical tests and high-performance liquid chromatography with a photodiode array. In further evaluations, antioxidant, antibacterial, antiviral, hen's egg test on chorioallantoic membrane (HET-CAM) assay, antipyretic, antiinflammatory, and antidiabetic activities were monitored. Using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, antioxidant potential was determined and maximum potential was observed in ethanol extracts. Antibacterial effects were confirmed through the disk diffusion method and minimum inhibitory concentrations were recorded against selected strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). In ovo antiviral activity of a viral inoculum (IBV or H9N2) was determined through *hemagglutination testing* and it was concluded that nearly all of the *C. myxa* extracts exhibited significant effects against the selected pathogenic strains of viruses and bacteria. To evaluate the toxicology, the HET-CAM assay was performed, revealing that the ethyl acetate and *n*-hexane extracts are nonirritating. The dichloromethane and ethyl acetate extracts were found to be the most effective in terms of in vivo pharmacological activities and antiinflammatory, antipyretic, and antidiabetic activities. Overall, the findings of this study suggest that *C. myxa* possesses substantial therapeutic potential and may serve as a valuable source of economical and efficacious alternative medicine.

Key words: *Cordia myxa*, antiviral, antiinflammatory, antipyretic, antidiabetic

1. Introduction

Cordia myxa, commonly known as Assyrian plum and referred to as lasura in Pakistan, is a medicinal plant belonging to the family Boraginaceae. It is found in tropical regions of Pakistan, India, and other South Asian countries (Abdel-Aleem et al., 2019). Phenols, terpenoids, alkaloids, nitrogen-containing compounds, phytosterols, and carotenoids are the major bioactive compounds of the genus (Marini et al., 2018). In *C. myxa*, the levels of unsaturated fatty substances are exceptionally high (9.9%). Significant amounts of minerals such as Na and K (13 and 29 ppm, respectively) were detected in edible parts of the plant with no presence of heavy metals, such as Pb, Cd, Cr, or Cu (Al-Khafaji et al., 2021). Over the past four decades, approximately 75% of natural compounds have undergone clinical trials, emphasizing the relevance of plant-derived therapies, and *C. myxa* offers particularly promising traditional therapeutic benefits, having antimicrobial, antifungal, analgesic, antibacterial, cytotoxic, gastroprotective, antiulcer, antiinflammatory, and wound-healing properties (Alibi et al., 2021). Recent studies revealed that medicinal plants of the Cholistan region are rich in many antibacterial agents (Shahzad et al., 2022a). Moreover, many medicinal plants have

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anthelmintic properties and are used to treat intestinal parasitosis of small ruminants (Degla et al., 2022).

Natural antioxidants are cost-effective as well as environmentally friendly. In the family Araucariaceae, *Araucaria heterophylla* is a well-known medicinal plant. The major component of this species is a carbohydrate-rich gum that possesses antioxidant, antimicrobial, antipyretic, neuroprotective, anticoagulant, and antiviral activities (Ashraf et al., 2023).Viral infections, including COVID-19, threaten human and animal life. To fight viral resistance, many researchers are exploring formulations of natural product-based antiviral agents (El Sayed, 2000). In response to inflammation, several inflammatory mediators including histamines, leukotrienes, chemokines, and cytokines are responsible for engaging leukocytes through chemo attraction (Arora and Ansari, 2019). Many new ayurvedic drugs have been discovered and several are in clinical trials to treat inflammation.

Pyrexia, or fever, entails an increase in internal heat level. It occurs as a result of both infectious and noninfectious agents (Niven and Laupland, 2016). As pathogens enter the body, pyrogens produced by the immune system give a signal to the hypothalamus to increase the body's temperature (Prajitha et al., 2018). PGE2 is a fever mediator in the brain formed by cycloxygenase-2 and microsomal PGE_2 synthase (Garami et al., 2018). Fever is a salient factor in many illnesses and disorders including contagion, aggravation, and injury (Gao et al., 2013).Yeast can produce and deliver endogenous pyrogens that can increase the internal body temperature. A yeast-initiated model of pyrexia is commonly used in pharmacological investigations of antipyretic impacts (Estella et al., 2022). A previous study revealed that the ethanol extract of *Atylosia rugosa* possesses antipyretic effects due to high concentrations of flavonoids and phenolic contents (Bharathi et al., 2022).

Diabetes mellitus (DM) is a multifactorial disease and a metabolic disorder, with up to 422 million new cases being reported every year (Rachpirom et al., 2022). Alloxan induction is one of the most effective ways to generate experimental DM. Alloxan is a urea subsidiary that causes the arrest of the β-cells of pancreatic islets in a particular way. Many pharmaceuticals have been introduced for treatment, but a complete cure for diabetes has not yet been discovered. Recent research has suggested that avocado seeds could activate the PI3K/AKT pathway and inhibit β-cell death, thus exhibiting antidiabetic potential (Ojo et al., 2022). Although *Cordia myxa* has long been used as a type of traditional medicine, there has been limited scientific validation of its pharmacological properties. Comprehensive analysis and in-depth investigations are still needed to identify the bioactive compounds that are responsible for the plant's pharmacological potential. Additionally, the plant's potential as a natural antiviral agent, particularly in the context of emerging viral threats such as COVID-19, remains largely unexplored. Thus, by conducting in vitro and in vivo studies, scientists should validate its therapeutic potential and mechanisms of action to develop plant-based drugs. The present study of *C. myxa* holds significant potential for advancing both traditional medicine and modern pharmacotherapy.

2. Materials and methods

2.1. Specimen collection

Fresh fruits and leaves of *C. myxa* were obtained from a village near the city of Bahawalpur, Pakistan (29.381199°N, 71.763480°E). This location was chosen due to its climatic and soil conditions as well as for ease of collection. After collection, the plant was identified by taxonomists from the Department of Botany of Islamia University of Bahawalpur and voucher number CM-192 was issued for further reference.

2.2. Preparation of extracts

Plant materials were dried in the shade for 3–4 weeks and ground into a fine powder. Subsequently, 10 g of dried plant was soaked in 100 mL of each solvent, including ethanol, methanol, ethyl acetate, dichloromethane, *n*-hexane, and an aqueous solvent. The mixtures were kept in air-tight containers for 3 days. Filtrates were dried in a rotary evaporator and preserved for further use.

2.3. Qualitative phytochemical analysis

For preliminary phytochemical analysis, the extracts were subjected to standard biochemical tests, including measurements of protein, carbohydrates, alkaloids, flavonoids, saponins, sterols, cholesterols, and phenols.

2.4. HPLC-PDA determination and chemical fingerprinting of phenols

High-performance liquid chromatography with a photodiode array (HPLC-PDA) and chemical fingerprinting of phenols were performed using the protocol of Marcello Locatelli as described by Zengin et al.(2016).

2.4.1. Chemicals and solvents

All chemical standards, including gallic acid, catechin, chlorogenic acid, *p*-OH benzoic acid, vanillic acid, epicatechin, syringic acid, 3-OH benzoic acid, 3-OH-4-MeO benzaldehyde, *p*-coumaric acid, rutin, sinapinic acid, *t*-ferulic acid, naringin, 2,3-diMeO benzoic acid, benzoic acid, *o*-coumaric acid, quercetin, harpagoside, *t*-cinnamic acid, naringenin, and carvacrol, were obtained from Sigma Aldrich (Milan, Italy). Methanol and acetonitrile (HPLC-grade), as well as acetic acid (99%), were obtained from Carlo Erba Reagents (Milan, Italy). Dimethyl sulfoxide (DMSO) was obtained from Honeywell (Tokyo, Japan). Milli-Q water was produced using a Millipore Milli-Q Plus water treatment system (Millipore Corp., Bedford, MA, USA).

2.4.2. Sample preparation

Samples for HPLC-PDA analysis were prepared as follows: plant extracts were weighed on an analytical balance and solubilized in mobile phase A (Milli-Q water + 3% acetic acid) and B (acetonitrile + 3% acetic acid) (93:7, v:v), adding 20% DMSO, except for the aqueous plant extracts, which were perfectly soluble in only the mobile phase. The samples were prepared at a concentration of 1 mg/250 µL. Each sample underwent vortexing for 30 s, followed by a 15-min sonication process. Subsequently, 20 µL of each prepared sample was injected into the HPLC system for analysis.

2.4.3. HPLC conditions

HPLC analyses were conducted following a previously validated method (Sotto et al., 2018) on a Waters liquid chromatograph equipped with a Model 600 solvent pump and a 2996 PDA, along with Empower v.2 Software (Waters Corporation, Milford, MA, USA), for data acquisition. The separation utilized a C18 reversed-phase packing column (Prodigy ODS (3), 4.6 × 150 mm, 5 µm; Phenomenex, Torrance, CA, USA), maintained at a temperature of 30 ± 1 °C using a Jetstream2 Plus column oven (UVISON Technologies Limited, England and Wales). The UV-Vis acquisition wavelength ranged from 200 to 500 nm. Quantitative analyses were performed at the maximum wavelength for each compound with an injection volume of 20 µL. The mobile phase, water-acetonitrile (93:7, v:v, 3% acetic acid), underwent online degassing using the Biotech DEGASi Compact (LabService, Anzoladell'Emilia, Italy).

2.5. In vitro assays

2.5.1. Antioxidant assays

2.5.1.1 Ferric reducing antioxidant power (FRAP) assay

To 20 µL of each sample (1 mg crude extract per 1 mL methanol), 90 µL of 0.2% PBS and 30 µL of 1% $K_3[Fe(CN)_6]$ were added. The microplate was incubated at 50 °C for 15 min. After adding 30 µL of 10% TCA and 30 µL of 0.1% $FeCl_3$ mixture held for 10 min, a bluish color appeared. Absorbance was measured at 700 nm. A positive control (ascorbic acid) and negative control (methanol) were also used.

2.5.1.2. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

In a 96-well ELISA plate, 30 µL of plant extract and 25 µL of DPPH were mixed and incubated for 10 min at 37 °C under dark conditions. The color change was noted and optical density was recorded at 517 nm. The assay was performed in triplicate. The percentage of radical scavenging activity (RSA) was calculated using the following formula:

$$\%RSA = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

IC_{50} values were calculated using GraphPad Prism software (GraphPad Inc., La Jolla, CA, USA).

2.5.2. Antibacterial activity

On nutrient agar media, 200 µL of freshly prepared bacterial culture (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) was applied and spread, and plates were incubated at 37 °C for 40 min. Disks presoaked in plant extracts along with positive (50 mg/mL ampicillin) and negative (normal saline) controls were applied to the culture plates under sterile conditions and incubated at 37 °C overnight. The zone of inhibition (ZoI) was measured. To determine the minimum inhibitory concentration (MIC), 50 µL of nutrient broth media and plant extracts were added up to the 12th well of the 96-well plate. After adding 100 µL of bacterial culture, incubation was performed at 37 °C for 24 h. Subsequently, 50 µL of INT solution prepared in methanol was added to each well. Color development indicated bacterial growth (Arshad et al., 2022).

2.6. In ovo antiviral assays

Viral strains were inoculated in specific pathogen-free embryonated chicken eggs of 9–11 days old via the chorioallantoic route. After incubation at 37 °C for 72 h, allantoic fluid was collected and the titer of each virus was determined through hemagglutination (HA) testing (Shahzad et al., 2022b).

2.6.1. Hemagglutination (HA) test

For the HA test, 50 µL of PBS was added to the 96-well plate, and 50 µL of viral inoculum (IBV or H9N2) was added to the 1st well and serially diluted up to the 11th well. The 12th well was left for the negative control (PBS). After the addition of 50 µL of 1% RBC solution in each well, the plate was finally incubated at 37 °C for 2–3 h. Red dots (button formation) at the bottom of wells reflected negative results, and a uniform reddish color in the wells signified a positive result. The IC_{50} of each positive extract was calculated via the serial dilution method, and 1:500 to 1:2500 dilutions of active compounds were made and tested in the HA test.

2.6.2. Hen's egg test on chorioallantoic membrane (HET-CAM) assay

Seven-day-old fertilized and viable white chicken eggs weighing approximately 50–60 g were used. Each test was applied to two eggs, with two as a standard treatment using 1% SDS and 0.1 M NaOH, and two as a control treated with 0.9% NaCl solution. To reduce the attachment of the embryo to the egg shell, eggs were incubated with the proper temperature (38.5–38.8 °C), relative humidity (60%–65%), and daily rotation. Air cells were marked. The part of the shell above the air cells was cut away using scissors. The eggs were then incubated again. Using tapered forceps, the membrane was carefully removed so as not to damage any underlying blood vessels. Eggs were only evaluated if the chorioallantois membrane had a noticeable fine vascular

system. Three endpoints were observed for 5 min in order to assess the irritating effect of 0.3 mL of the tested items: coagulation (intra- and extravascular denaturation of protein), lysis (disintegration of vessels), and hemorrhage (bleeding from vessels).

2.7.α-Glucosidase inhibition assay

The substrate for the inhibition assay was p-nitrophenyl-α-D-glucopyranoside (p-NPG); the enzyme α-glucosidase is produced from yeast. Acarbose served as the experiment's positive control, and 0.04 units/mL enzyme and 0.7 mM substrate were taken for the first screening for the plant extracts. A 100 mM buffer of Na_3PO_4 with 50 mM NaCl was prepared in demineralized water of pH 6.8. Using a microtiter plate reader, the change in absorbance at 405 nm as a result of the hydrolysis of p-NPG by α-glucosidase was measured in 96-well plates. There was an increase in absorbance at 405 nm because of the action of an enzyme hydrolyzing p-NPG to form the p-nitrophenolate ion. The experiment was conducted with the temperature maintained at 37 °C. Prior to incubation and 30 min later (after the addition of p-NPG substrate), the absorbance was measured twice.

2.8. In vivo assays

2.8.1. Experimental animals and ethical approval

Healthy albino female rats weighing between 150 and 250 g were used in this study. Animals were kept in the Animal House Facility of the Department of Food Sciences of The Islamia University of Bahawalpur. All animals were placed under standard laboratory conditions of a 12-h light/dark cycle at 22 ± 1 °C with 35–60 ± 5% humidity, unrestricted access to standard food, and unlimited access to water for at least 1 week before the experiment.

All procedures were conducted in accordance with the relevant guidelines for the use and care of laboratory animals (PAEC/20/18, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan). The protocols were approved by the Institutional Committee for the Care and Use of Laboratory Animals. We took special care to minimize animal suffering and the number of animals used.

2.8.2. Acute toxicity study

An acute toxicity investigation of *C. myxa* fruit extracts was carried out on female albino rats weighing 150–250 g. Normal saline was given to the control group as a treatment. Seven groups of 3 animals were formed and administered dosages of 0, 200, 500, 1000, 2000, 3000, and 5000 mg/kg. Animals were monitored for 6 h for any acute symptoms of toxicity or death before administering large dosages. Every dosage was administered orally. Under standard atmosphere settings, with free access to food and water, toxicity indications and behavioral abnormalities such as hyperactivity, convulsions, corneal reflex, perspiration, alertness, urine changes, and death were observed for 24 h.

2.8.3. Antiinflammatory activity

Female albino rats weighing 150–250 g were used to evaluate the antiinflammatory potential of *C. myxa* fruit extract. Animals were divided into 9 groups of 3 animals, and 0.1 mL of carrageenan solution (1% v/w) was injected into the right hind paw of the animals to induce inflammation. Diclofenac sodium (15 mg/kg) was used as a positive control and normal saline was used as a negative control. Only two doses (400 and 500 mg/kg) were tested orally 30 min after the carrageenan injection. Paw size was measured at 0, 1, 2, and 3 h after treatment using a Vernier caliper (Jisha et al., 2019). The percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control mean} - \text{treated mean} \times 100}{\text{Control mean}}$$

2.8.4. Antipyretic activity

Albino female rats weighing 150–230 g were used. Different crude extracts of *C. myxa* fruit were used to evaluate antipyretic activity with a yeast-induced pyrexia protocol. The animal's rectal temperature was measured carefully with a digital thermometer, and a subcutaneous injection of 10% Brewer's yeast solution was used to cause pyrexia. Paracetamol (150 mg/kg) was used as a positive control and normal saline was used as a negative control. Eleven groups of 3 animals were formed. Only two doses (400 and 500 mg/kg) were tested orally after initiating pyrexia. Each animal's rectal temperature was noted at 0, 1, 2, and 3 h after treatment (Abdel-Aleem et al., 2019).

2.8.5. Antidiabetic activity

The antidiabetic potential of *C. myxa* fruit extracts was tested in alloxan-induced rats. Intraperitoneal injections of alloxan monohydrate (120 mg/kg) were administered. One hour after the alloxan injection, the animals received food and a 5% dextrose solution to control early hypoglycemic conditions. After 72 h, blood glucose levels were monitored using a glucometer. Diabetic rats (sugar level of >150 mg/dL) were divided into 6 groups of 4 animals. Glibenclamide (5 mg/kg) was used as a positive control and normal saline was used as a negative control, and a crude extract dose of 500 mg/kg was given orally to the animals for 7 days (Raju and Hemamalini, 2012). The percentage of change in blood glucose level was calculated using the following formula (Abdel-Aleem et al., 2019):

$$\% \text{ lowering of blood glucose} = \frac{\text{Wc (fasting)} - \text{Wt (test)} \times 100}{\text{Wc (fasting)}}$$

2.9. Statistical analysis

All data were presented as mean ± standard error of the mean. Statistical analysis was performed using t-tests and one-way analysis of variance (ANOVA) to compare the

means among various groups. Values of $p < 0.05$ were considered statistically significant. Graphical illustrations were prepared using Microsoft Excel.

3. Results

3.1. Phytochemical analysis

The crude extracts of *C. myxa* were subjected to phytochemical tests and the results are shown in Table 1. The results indicated that alkaloids, flavonoids, proteins, and carbohydrates were highly present in the aqueous, ethanol, and methanol extracts. *n*-Hexane extracts exhibited high quantities of saponins and steroids (Table 1).

3.2. HPLC-PDA determination and chemical fingerprinting of phenols

According to HPLC-PDA analysis, different phenolic compounds were observed in the *n*-hexane, methanol, aqueous, ethanol, and ethyl acetate extracts. In methanol and ethanol leaf extracts, catechin was recorded at 10.46 ± 0.52 and 0.59 ± 0.04, respectively. In the ethanol leaf extract, chlorogenic acid (0.74 ± 0.04) and 2,3-diMeO benzoic acid (0.72 ± 0.05) were also observed. In the ethyl acetate fruit extract, naringenin (0.31 ± 0.02) was recorded. During HPLC-PDA analysis, some compounds were found to be below the limit of quantification (BLQ) (Table 2).

Table 1. Phytochemical analysis of crude extracts of *C. myxa* leaves (L) and fruits (F).

Test type	EtOH		MtOH		EA		*n*-Hex		Aq	
Saponins test	L	F	L	F	L	F	L	F	L	F
	-	+	-	-	-	+	+	++	-	++
Millon's test	++	++	++	++	+	+	+	++	++	-
Flavonoids test	-	-	-	-	-	++	+	++	-	-
Phenols	-	-	-	-	+	-	-	-	-	+
Liberman's test	-	+	-	+	-	+	++	++	-	-
Terpenoids	-	-	-	-	-	++	++	-	-	-
Benedicts test	++	-	++	-	++	-	-	-	++	-
Salkowski's test	++	++	++	++	++	++	++	++	++	++
Ninhydrin	-	-	-	-	++	-	-	-	-	-

(*- not detected, + Slightly present, ++ Highly present)

Table 2. HPLC-PDA analysis and chemical fingerprinting of phenols.

Phenols	n-hexane	Aqueous	methanol	Aqueous	ethanol	Ethyl acetate
Gallic acid Catechin			(10.46 ± 0.52)L		(0.59 ± 0.04) L	BLQ
Chlorogenic acid					(0.74 ± 0.04) L	(0.5± 0.34)
p-OH benzoic acid		BLQ	BLQ	BLQ (>LOD)	BLQ	
Vanillic acid			BLQ		BLQ	
3-OH-4-MeO benzaldehide						BLQ
p-coumaric acid					BLQ	BLQ
Sinapinic acid			BLQ		BLQ	BLQ
t-ferulic acid					BLQ	
Naringin					(0.26± 0.3)F	(0.31 ± 0.02) F
2,3-diMeO benzoic acid					(0.72 ± 0.05) L	
Benzoic acid			(1.57 ± 0.1)F			
o-coumaric acid					BLQ	

***L=Leaves *F=Fruits**

3.3. In vitro assays

3.3.1. Antioxidant potential

According to the FRAP assay results, the methanol leaf extract and ethanol fruit extract had the maximal antioxidant potential. The overall trend in the FRAP assay for different extracts of *C. myxa* leaves was as follows: methanol > ethanol > *n*-hexane > ethyl acetate > aqueous. For the fruit extracts, this order was ethanol > methanol > *n*-hexane > aqueous > ethyl acetate.

For DPPH activity, maximal inhibition of 38.63% was shown by the ethanol fruit extract compared to the positive control. Compared to the control, other extracts showed less antioxidant activity. The overall trends for *C. myxa* fruit and leaf extracts for DPPH activity were respectively ethanol > aqueous > methanol > ethyl acetate > *n*-hexane and ethanol > *n*-hexane > methanol > ethyl acetate > aqueous. Values of %RSA are shown in Figure 1.

3.3.2. Antibacterial activity

Antibacterial activity studies revealed that almost all extracts were active against different bacterial strains but in varying orders. The maximum ZoI of 25 ± 0.06 was obtained for the *n*-hexane leaf extract against *P. aeruginosa*. For fruit extracts, the maximum inhibition was obtained for methanol (20 ± 0.05) against *P. vulgaris*. Antibacterial studies further revealed that *C. myxa* fruit extracts had more potential than leaf extracts. In the case of fruit extracts, the order of activity of solvents against different microbes was methanol > ethyl acetate > ethanol > aqueous > *n*-hexane. In the case of leaf extracts, the overall trend was *n*-hexane > ethyl acetate > ethanol > methanol > aqueous (Tables 3 and 4).

3.3.3. Antiviral activity

C. myxa extracts were tested against the two most common poultry viruses: AIV-H_9N_2 and IBV. Results

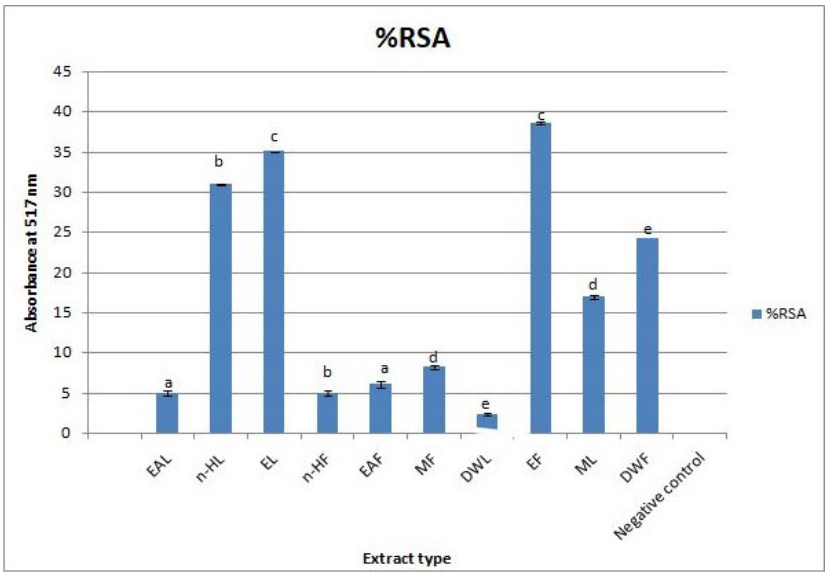


Figure 1. Antioxidant activity of methanol, ethanol, ethyl acetate, *n*-hexane, and aqueous extracts of leaves and fruits of *C. myxa* as revealed by DPPH assay.

Table 3. Minimum inhibitory concentration (/µL) and zone of inhibition (mm) for *C.myxa* leaves against different strains of bacteria.

Extract types	*MDR S.aureus*		*MDR P.aeruginosa*		*E.coli*		*S.aureus*		*P.vulgaris*		*K. pneumoniae*		*P.aeruginosa*	
	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC
MtOH	6±0.5	-	6±0	-	6±1.25	-	6±0.57	-	6±0.54	-	6±1.75	-	10±0.05	6.25
EtOH	7±0.57	-	6±0.53	-	6±1.25	-	7±0	25	11±0	25	6±0	-	10±1.75	6.25
Aq	7±0.06	-	9±0.45	6.25	6±0.05	-	7±0.07	-	6±1.25	-	7±0.05	-	6±1.75	-
EA	6±0.5	-	6±0.43	-	6±0.08	-	6±1.4	-	6±0	-	6±0.06	-	19±0.07	12.5
n-Hex	10±0.02	6.25	6±0.05	-	8±0	6.25	6±1.05	-	6±1.75	-	6±0.05	-	25±0.06	-

Table 4. Minimum inhibitory concentration (/µL) and zone of inhibition (mm) for *C. myxa* fruit against different strains of bacteria.

Extract types	*MDR S.aureus*		*MDR P.aeruginosa*		*E.coli*		*S.aureus*		*P.vulgaris*		*K.pneumoniae*		*P.aeruginosa*	
	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC	ZoI±SEM	MIC
MtOH	7±0.06	-	7±0.24	0.78	7±1.02	-	8±0.02	6.25	20±0.05	1.56	6±1.05	-	6±0.75	-
EtOH	10±0.02	3.125	8±1.075	0.78	7±0.08	-	6±0.14	-	11±0.16	-	11±0.01	12.5	11±0.01	3.125
Aq	10±0	6.25	6±1.06	-	6±0.04	-	6±0.078	-	10±0	12.5	10±0.05	12.5	10±0.04	-
EA	6±0	-	6±0.04	-	6±0.02	-	8±0.4	25	15±0.4	1.56	6±0.02	-	6±0.06	-
n-Hex	10±1.04	6.25	6±1.04	-	8±0.02	3.12	7±0.02	-	9±0.06	6.25	6±1.06	-	9±1.07	-
+ve control	19±1.47	0.78	12±0.02	0.78	12±0	6.25	18±1.35	25	17±1.76	3.12	16±0.45	25	19±0.39	6.25

showed that in leaf extracts, excluding the ethyl acetate extract, all had maximum potential with HA titers of 0 (log_{10} reduction = 100%) against AIV-H_9N_2. Against IBV, aqueous leaf extracts exhibited the highest potential. For fruit, aqueous extracts also exhibited the highest antiviral potential against selected viral strains (Table 5).

3.3.4. HET-CAM assay

HET-CAM tests were performed were aimed to determine the toxicology in a chicken's chorioallantoic membrane. The commencement of bleeding, coagulation, and vessel lysis was used to gage the effects. Toxicological observations were made after 0.5, 1, 2, and 5 min. Results revealed that the *n*-hexane and ethyl acetate extracts were nonirritating, while dichloromethane and methanol were moderate irritants because after 5 min and 4.5 min, respectively, lysis was observed (Table 6; Figure 2).

3.3.5. α-Glucosidase inhibition assay

Glucosidase inhibition assay results for all plant extracts of *C. myxa* fruit were noticeably good. Ethyl acetate had the highest glucosidase activity of 91.50%, followed by the dichloromethane extract at 83.60% and then *n*-hexane at 73%. The methanol extract had minimum inhibition of 46.80%. The overall activity trend was ethyl acetate > dichloromethane > *n*-hexane > methanol (Figure 3).

3.4. In vivo activities

3.4.1. Acute toxicity

Acute toxicity assays showed no major complications or mortality up to the 5000 mg/kg dosage level, so it was concluded that *C. myxa* is safe to use (Amarasiri et al., 2020).

3.4.2 Antiinflammatory activity

Antiinflammatory activity results showed that diclofenac sodium as the positive control exhibited 26.57% inhibition against inflammation. The maximum inhibition was achieved with dichloromethane at 22.5% and minimum inhibition was achieved with ethyl acetate at 17.06% compared to the positive control. All extracts displayed dose-dependent activity. Further statistical analysis was conducted, indicating significant differences in the efficacy of dichloromethane extracts compared to the positive control groups at both doses ($p < 0.05$). Additionally, statistical significance was obtained for the lower activity observed with the ethyl acetate extract ($p < 0.05$).The overall trend of *C. myxa* fruit extracts in inhibiting inflammation was dichloromethane > methanol > *n*-hexane > ethyl acetate (Figure 4).

3.4.3. Antipyretic activity

The antipyretic potential of *C. myxa* fruit extracts was observed using a yeast-induced pyrexia method. The ethyl acetate extracts of *C. myxa* showed maximum antipyretic potential. Highly significant results were obtained after 1 h and maintained for up to 3 h for the ethyl acetate extract at a dose level of 500 mg/kg, with antipyretic potential equal to that of the positive control (paracetamol). Statistical analysis showed the significant potential of *C. myxa* fruit extracts against pyrexia ($p < 0.05$). The overall trend was ethyl acetate > dichloromethane > methanol > *n*-hexane (Figure 5).

3.4.4. Antidiabetic activity

Antidiabetic activity tests showed that the dichloromethane extract exhibited the maximum effect (16%) in lowering blood glucose and that the *n*-hexane extract had minimum potential (7.3%) compared to the controls. *n*-Hexane yielded nonsignificant results on the 1st and 3rd days, but on the 5th and 7th days it achieved low positive results. Comprehensive statistical analysis revealed significant differences among the groups ($p < 0.05$).The overall trend for antidiabetic activity was dichloromethane > ethyl acetate > methanol > *n*-hexane (Figure 6).

4. Discussion

This study was designed to evaluate the in vitro and in vivo activities and perform phytochemical analysis of *C. myxa*. In phytochemical analysis, numerous compounds such as saponins, carbohydrates, proteins, alkaloids,

Table 5. Antiviral activity of *C. myxa* leaves and fruits.

Plant name	Extract type	AIV-H9N2		IBV	
		HA titer	IC_{50}	HA titer	IC_{50}
C. myxa (fruit)	EtOH	2	12.5	4	12.5
	MtOH	4	6.25	0	12.5
	Aq	0	6.25	0	6.25
	EA	4	12.5	8	25
	n-Hex	0	12.5	4	12.5
C. myxa (leaves)	EtOH	0	6.25	0	6.25
	MtOH	0	6.25	0	6.25
	Aq	0	12.5	2	12.5
	EA	8	25	0	6.25
	n-Hex	0	6.25	0	12.5
Positive control		-	-	-	-
Virus control/negative control		1024	-	1024	-

Table 6.HET-CAM assay results for *Cordia myxa*.

Extracts	Hemorrhage	Lysis	Coagulation	Category
MtOH	2 min	4.5 min	0	**Slightly irritant**
***n*- Hex**	2 min	0	0	**Nonirritant**
DCM	1.5 min	5 min	0	**Moderately irritant**
EtOAc	1 min	0	0	**Nonirritant**

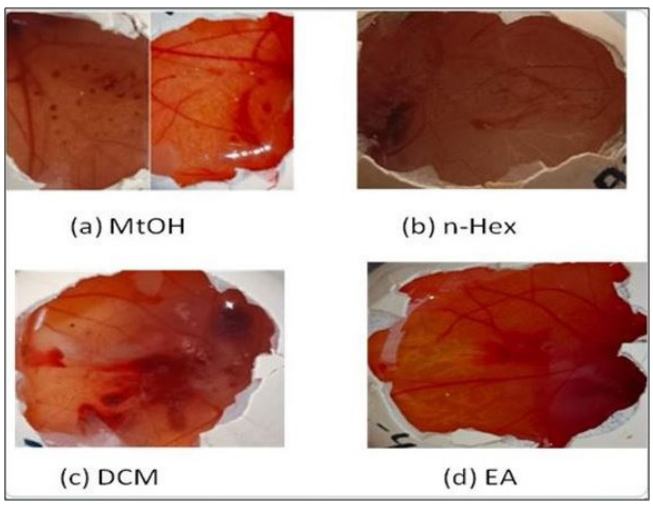


Figure 2. HET-CAM assay results for *Cordia myxa* fruit extracts.

terpenoids, glycosides, cholesterols, flavonoids, sterols, and phenols were detected. Past studies identified 290 phytoconstituents of various classes within the genus *Cordia* (Nariya et al., 2013). Reactive oxygen species (ROS) may cause damage inside the cells of an organism. Natural antioxidants can eliminate or neutralize those ROS. In this study, an antioxidant assay was performed using the FRAP and DPPH methods. The results revealed that overall, both the fruit and leaves of *C. myxa* are good sources of antioxidants. In FRAP and DPPH antioxidant assays, the maximum potential was shown by ethanol fruit extracts and the minimum potential was shown by aqueous leaf extracts of *C. myxa*. The maximum potential may have been due to the presence of terpenoids, flavonoids, phenols, and polyphenols, which are medicinal plant-based free radical scavengers (Nwozo et al., 2023). Previous studies

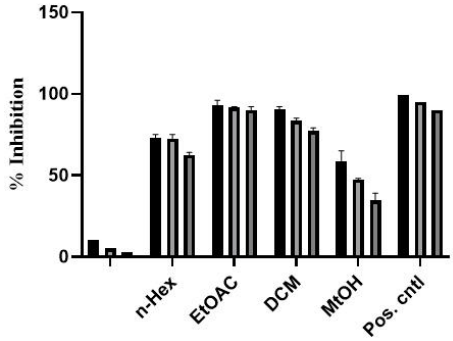
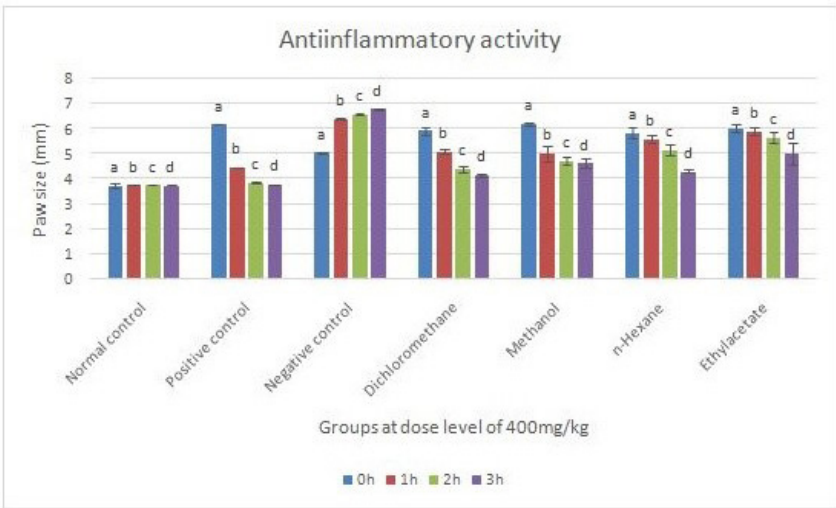
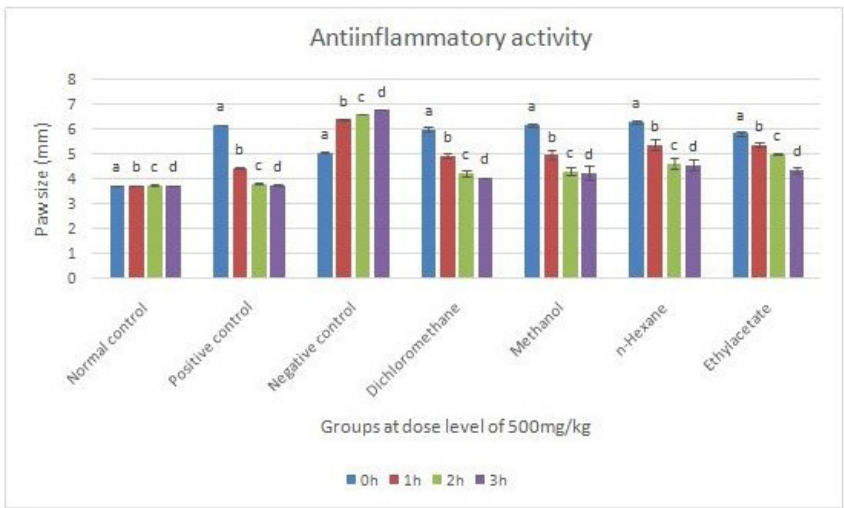


Figure 3.α-Glucosidase inhibition assay results for *Cordi amyxa*.



(a)



(b)

Figure 4. Antiinflammatory activity of *C. myxa* fruit extracts. Each value represents a mean value ± standard error of the mean (n = 5). Significance was evaluated at $p < 0.05$ using t-tests.

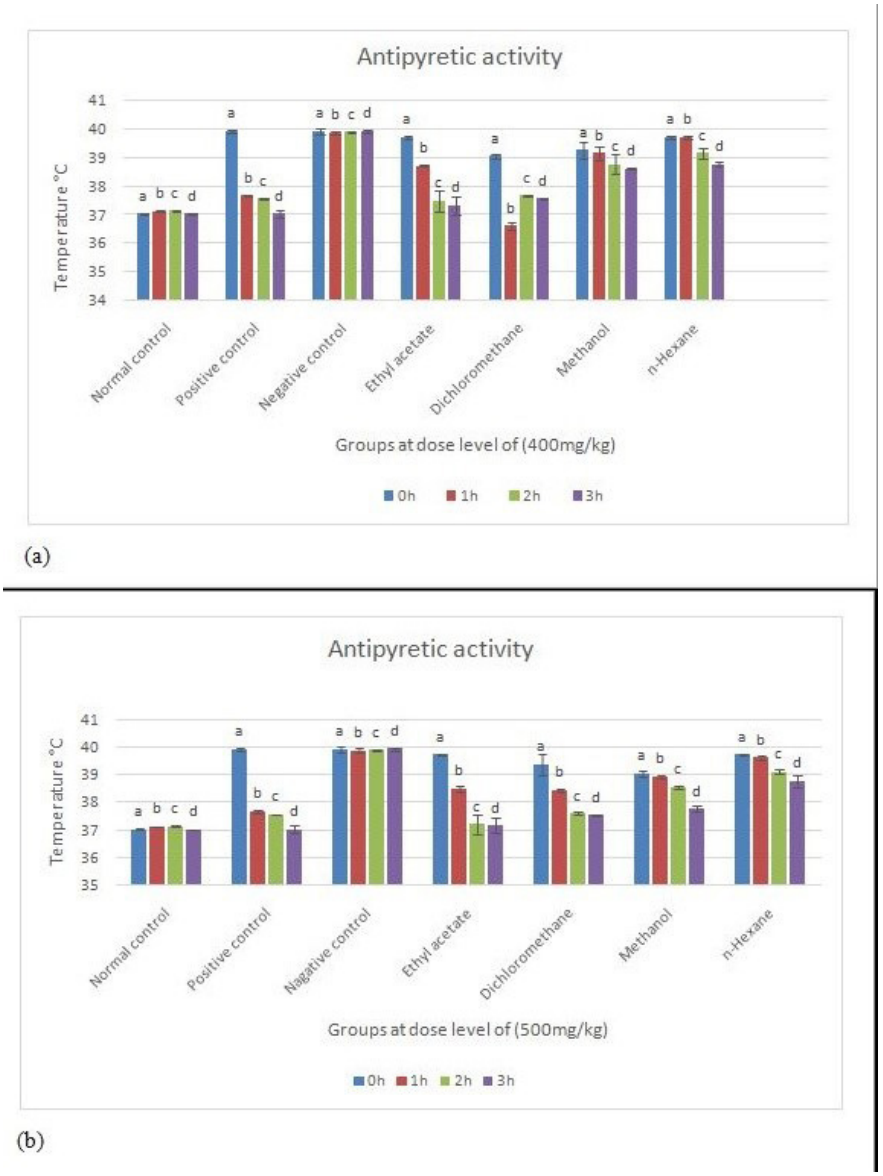


Figure 5. Antipyretic activity of *C. myxa* fruit extracts. Each value represents a mean value ± standard error of the mean (n = 5). Significance was evaluated at $p < 0.05$ using t-tests.

revealed that phenols are the best scavengers of ROS. They are directly involved in antioxidant action (Al-Musawi et al., 2022).

Antibacterial activity was evaluated to identify potential antibacterial drugs from among the crude extracts of the plant. During this analysis, it was found that the *C. myxa* fruit extracts had more potential for antibacterial activity than leaf extracts. These results suggest that *C. myxa* extracts can be applied against foodborne illnesses caused by *E. coli* and *S. aureus* (Yaermaimaiti et al., 2021). In *C. myxa* fruit extracts, many phenolic compounds are of great value for their antimicrobial potential. These extracts may be used for food preservation due to their high potential to prevent bacterial growth (Ghildiyal et al., 2020). In the analysis of antiviral activity, all extracts of *C. myxa* were seen to be strongly active against H9N2 (AIV) and IBV viral strains. The ethanol and methanol leaf extracts had the highest antiviral activity, while the ethyl acetate leaf and fruit extracts had the least antiviral potential. Several secondary metabolites inhibit viral transcription or replication, including coumarins, terpenes, terpenoids, and flavonoids (Monte et al., 2014). These secondary metabolites are involved in the disruption of microbial cell membranes and cell walls as well as various other biological processes, ultimately leading to the inhibition of microbial growth (Meyer et al., 2019).

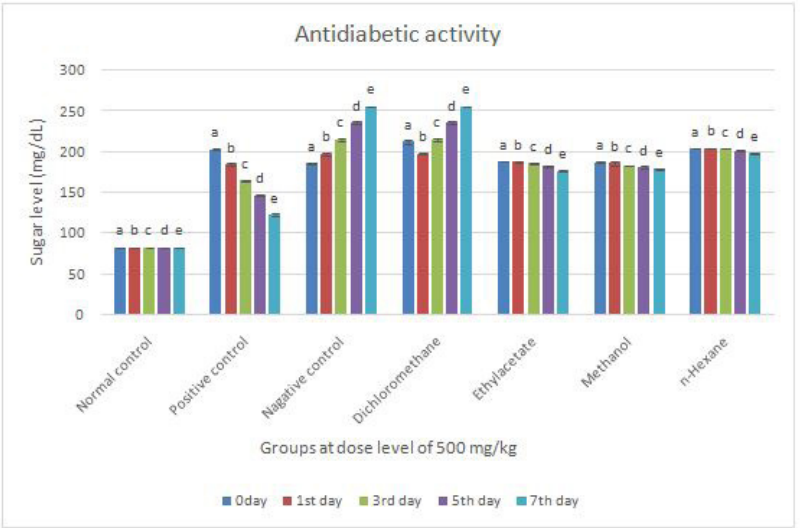


Figure6. Antidiabetic activity of *C. myxa* fruit extracts. Each value represents a mean value ± standard error of the mean (n = 5). Significance was evaluated at $p < 0.05$ using t-tests.

HET-CAM assay tests were performed to determine the toxicology in a chicken's chorioallantoic membrane. Different extracts showed different levels of irritation during the tests. Some extracts were strong irritants, like methanol, while some, like dichloromethane, were moderate irritants. Ethyl acetate and *n*-hexane were found to be nonirritating. Similarly, the anthelmintic properties of various compounds were previously studied (Ahmad et al., 2023). *C. myxa* extracts showed considerable potential against pathogenic infections without causing toxicity, as confirmed by the HET-CAM assay.

A carrageenan-induced rat model was used to evaluate the antiinflammatory activity of *C. myxa* fruit extracts. Carrageenan causes inflammation by stimulating the TLR4 signaling pathway, an inflammation-inducing pathway. The dichloromethane extract of *C. myxa* fruit showed maximum results against inflammation. Doses of 400 and 500 mg/kg were both highly effective. This may be attributed to the high contents of flavonoids and inhibition of the COX and 5LOX pathways, decreasing eicosanoid synthesis together with the capability to impact neutrophil levels (Raju and Hemamalini, 2012). Polyphenols, alkaloids, and flavonoids are thought to be particularly responsible for encouraging antiinflammatory activities. In a previous study, *Cordia dichotoma* was reported to have antiinflammatory potential due to its tannins, flavonoids, alkaloids, glycosidic compounds, saponins, and carbohydrates (Younus and Siddiq, 2022).

A pyrexia model induced by yeast was used to evaluate the antipyretic potential of *C. myxa* fruit extracts. The ethyl acetate and dichloromethane extracts of *C. myxa* showed highly significant results. Phytochemical screening of *C. myxa* fruit extracts revealed that phenols, flavonoids, and glycosides are present in the ethyl acetate extract at significant levels. The presence of tannins was also confirmed. Potent pyrexia inhibition may be due to the presence of sterols and flavonoids (Raju and Hemamalini, 2012). Studies have also reported that flavonoids and plant-derived secondary metabolites are involved in the inhibition of prostaglandins (Younus and Siddiq, 2022). This may suggest that flavonoids are responsible for the antipyretic activity, but in the ethyl acetate and methanol extracts, phenols and glycosides are also present in high amounts. Thus, they may also be responsible for the reduction of fever, but this requires further studies for confirmation.

α-Glucosidase inhibition is a critical mechanism in reducing glucose absorption in the bloodstream, thereby helping to regulate blood sugar levels. *C. myxa* fruit extracts yielded promising results in this regard. Among the tested extracts, ethyl acetate exhibited the highest α-glucosidase inhibitory activity, followed by dichloromethane. In contrast, the methanol extract showed the lowest inhibition activity, while *n*-hexane ranked third in terms of efficacy. An in vivo antidiabetic trial revealed that the dichloromethane and ethyl acetate extracts exerted significant activities in maintaining the blood glucose level, in contrast to the *n*-hexane extract. The actions of flavonoids, tannins, phenolics, and alkaloids against β-cells to produce insulin have been noted in this context. It was also reported that these phytochemicals are responsible for increasing insulin sensitivity; thus, they may be responsible for reducing diabetes (Alibi et al., 2021).

While the present study focused on the phytochemical analysis and pharmacological activities of *Cordia myxa*, advances in technologies such as next-generation sequencing

offer exciting opportunities for further exploration of the plant's genetic makeup (Ali et al., 2023), phytochemical production, and potential for breeding new varieties with enhanced medicinal qualities. Moreover, employing metabolomic tools such as GC-MS, LC-MS, CE-MS, and NMR (Noman and Azhar, 2023) for the metabolic profiling of *C. myxa* could offer valuable insights into how the plant adapts to harsh environmental stresses, potentially affecting its pharmacological properties.

In conclusion, *C. myxa* has significant therapeutic properties, particularly in terms of its antiinflammatory, antidiabetic, and antioxidant activities, making it a promising source of alternative medicine. The ethyl acetate and dichloromethane extracts were most effective. These findings show that progression from preclinical to clinical trials is warranted to further explore its medicinal value. Additionally, *C. myxa* holds interdisciplinary relevance in nutrition as a potential functional food, in agriculture for sustainable cultivation, and in sustainability by providing a natural, environmentally friendly alternative to synthetic drugs. Continued research could open new avenues for pharmaceutical applications.

Conflict of interest

There is no conflict of interest.

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References

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

Al-Khafaji SA, Alsaadawi MA, Al-Yasari AM, Al-Saadawe MA (2021). Article Review: Cordia myxa L.: The gift of nature, A Review. Basrah Journal of Agricultural Sciences 34 (2): 267-277. https://doi.org/10.37077/25200860.2021.34.2.20

Alibi S, Crespo D, Navas J (2021). Plant-derivatives small molecules with antibacterial activity. Antibiotics 10: 231. https://doi.org/10.3390/antibiotics10030231

Al-Musawi MH, Ibrahim KM, 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

Amarasiri SS, Attanayake AP, Jayatilaka KAW, Mudduwa LKB (2020).South Asian medicinal plants and chronic kidney disease. Traditional Medicine Research 5 (5): 389-412. https://doi.org/10.53388/tmr20200603189

Arora P, Ansari SH. (2019). Role of various mediators in inflammation of asthmatic airways. In: Pereira C (editor). Asthma - Biological Evidences. Intech Open 1 (14): 95-104. https://doi.org/10.5772/intechopen.84357

Arshad M, Ruby T, Shahzad MI, Alvi Q, Aziz M et al. (2022). An antimicrobial activity of oil extracted from Saarahardwickii. Brazilian Journal of Biology 84 (1): 1-7. https://doi.org/10.1590/1519-6984.253508

Ashraf M, Ahmad N, Akbar F, Fazal H, Ali L et al. (2023). Time and concentration-dependent differential antioxidant potential in the gum of medicinally important Araucaria heterophylla. Agrobiological Records 13 (1): 44-52. https://doi.org/10.47278/journal.abr/2023.024

Ahmad S, Humak F, Ahmad M, Altaf H, Qamar W et al. (2023). Phytochemicals as alternative anthelmintics against poultry parasites: a review. Agrobiological Records 12 (1): 34-45. https://doi.org/10.47278/journal.abr/2023.015

Ali K, Ghous HF, Shehzadi N, Haroon O, Rashid S et al. (2023). Exploring the potential of next generation sequencing in plant breeding and genetics. Agrobiological Records 11 (1): 1-5. https://doi.org/10.47278/journal.abr/2023.001

Bharathi PM, Alagarsamy V, Prasad SS, Vali SC, Krishna VM. (2022). Phytochemical Screening and Antipyretic activity of Atylosia rugosa. Research Journal of Pharmacy and Technology. 15 (2): 701-716. https://doi.org/10.52711/0974-360x.2022.00116

Degla LH, Kuiseu J, Olounlade PA, Attindehou S, Hounzangbe-Adote MS et al. (2022). Use of medicinal plants as alternative for the control of intestinal parasitosis: assessment and perspectives. Agrobiological Records 7 (1): 1-9. https://doi.org/10.47278/journal.abr/2021.011

Di Sotto A, Checconi P, Celestino I, Locatelli M, Carissimi S et al. (2018). Antiviral and antioxidant activity of a hydroalcoholic extract from *Humulus lupulus* L. Oxidative Medicine and Cellular Longevity 2018 (1): 5919237. https://doi.org/10.1155/2018/5919237

El Sayed KA (2000).Natural products as antiviral agents. In: Atta-ur-Rahman (editor). Studies in Natural Products Chemistry. Elsevier 24 (1): 473-572. https://doi.org/10.1016/s1572-5995(00)80051-4

Estella OU, William AC, Patrick O, Ikenna C, Mba T et al. (2022). Evaluation of the analgesic and antipyretic activity of methanol extract of *Combretum bauchiense* Hutch & Dalziel (Combretaceae) leaves. Phytomedicine Plus 2 (1): 100166. https://doi.org/10.1016/j.phyplu.2021.100166

Gao X, Guo M, Zhao B, Peng L, Su J et al. (2013). A urinary metabonomics study on biochemical changes in yeast-induced pyrexia rats: a new approach to elucidating the biochemical basis of the febrile response. Chemico-Biological Interactions 204 (1): 39-48. https://doi.org/10.1016/j.cbi.2013.04.001

Garami A, Steiner AA, Romanovsky AA (2018). Fever and hypothermia in systemic inflammation. In: Romanovsky AA (editor). Handbook of Clinical Neurology 157: 565-597. https://doi.org/10.1016/b978-0-444-64074-1.00034-3

Ghildiyal R, Prakash V, Chaudhary VK, Gupta V, Gabrani R (2020). Phytochemicals as antiviral agents: recent updates. In: Swamy M (editor). Plant-Derived Bioactives. Springer 1 (1): 279-295. https://doi.org/10.1007/978-981-15-1761-7_12

Jisha N, Vysakh A, Vijeesh V, Latha MS (2019). Anti-inflammatory efficacy of methanolic extract of *Muntingia calabura* L. leaves in carrageenan-induced paw edema model. Pathophysiology 26 (1): 323-330. https://doi.org/10.1016/j.pathophys.2019.08.002

Monte J, Abreu AC, Borges A, Simões LC, Simões M (2014). Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. Pathogens 3 (2): 473-498. https://doi.org/10.3390/pathogens3020473

Marini G, Graikou K, Zengin G, Karikas GA, Gupta MP et al. (2018). Phytochemical analysis and biological evaluation of three selected Cordia species from Panama. Industrial Crops and Products 120 (1): 84-89. https://doi.org/10.1016/j.indcrop.2018.04.037

Meyer MF, Powers SM, Hampton SE (2019). An evidence synthesis of pharmaceuticals and personal care products (PPCPs) in the environment: imbalances among compounds, sewage treatment techniques, and ecosystem types. Environmental Science & Technology 53 (22): 12961-12973. https://doi.org/10.1021/acs.est.9b02966

Nwozo OS, Effiong EM, Aja PM, Awuchi CG (2023). Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review. International Journal of Food Properties 26 (1): 359-388. https://doi.org/10.1080/10942912.2022.2157425

Nariya PB, Bhalodia NR, Shukla VJ, Acharya R, Nariya MB (2013). In vitro evaluation of antioxidant activity of *Cordia dichotoma* (Forst f.) bark. Ayu 34 (1): 124. https://doi.org/10.4103/0974-8520.115451

Niven DJ, Laupland KB. (2016). Pyrexia: aetiology in the ICU. Critical Care 20 (1): 1-9. https://doi.org/10.1186/s13054-016-1406-2

Noman MU, Azhar S (2023). Metabolomics, a potential way to improve abiotic stresses tolerance in cereal crops. International Journal of Agriculture and Biosciences 12 (1): 47-55. https://doi.org/10.47278/journal.ijab/2023.043

Ojo OA, Amanze JC, Oni AI, Grant S, Iyobhebhe M et al. (2022). Antidiabetic activity of avocado seeds (*Perse americana* Mill.) in diabetic rats via activation of PI3K/AKT signaling pathway. Scientific Reports 12 (1): 2919. https://doi.org/10.1038/s41598-022-07015-8

Prajitha N, Athira S, Mohanan P (2018). Pyrogens, a polypeptide that produces fever by metabolic changes in hypothalamus: mechanisms and detections. Immunology Letters 204 (1): 38-46. https://doi.org/10.1016/j.imlet.2018.10.006

Rachpirom M, Barrows LR, Thengyai S, Ovatlarnporn C, Sontimuang C et al. (2022). Antidiabetic activities of medicinal plants in traditional recipes and candidate antidiabetic compounds from *Hydnophytum formicarum* Jack. Tubers. Pharmacognosy Research 14 (1): 89-99. https://doi.org/10.5530/pres.14.1.13

Raju S, Hemamalini K (2012). In vivo animal model for screening of anti-diabetic activity. Biosciences Biotechnology Research Asia 9 (1): 765-772. https://doi.org/10.13005/bbra/1062

Shahzad MI, Anwar S, Aslam J, Manzoor A, Ashraf H et al. (2022a). Antibacterial and antibiofilm activities from extracts of selected Cholistani plants. Frontiers in Chemical Sciences 3 (1): 32-45. https://doi.org/10.52700/fcs.v3i1.50

Shahzad MI, Ahmed M, Asghar N, Ashraf H, Anwar S et al. (2022b). In ovo antiviral screening of Cholistani plants against Swine influenza virus and confirmation through real-time PCR. Frontiers in Chemical Sciences 3 (1): 79-89. https://doi.org/10.52700/fcs.v3i1.49

Younus I, Siddiq A (2022). *Raphanussativus* L. var. *caudatus* as an analgesic and antipyretic agent in animal models. Pakistan Journal of Zoology 54 (1): 1643. https://doi.org/10.17582/journal.pjz/20200812110845

Yaermaimaiti S, Wu T, Aisa HA (2021).Bioassay-guided isolation of antioxidant, antimicrobial, and antiviral constituents of Cordiadichotoma fruits. Industrial Crops and Products 172 (1): 113977. https://doi.org/10.1016/j.indcrop.2021.113977

Zengin G, Locatelli M, Ceylan R, Aktumsek A (2016). Anthraqeousuinone profile, antioxidant, and enzyme inhibitory effect of root extracts of eight Asphodeline taxa from Turkey: Can Asphodeline roots be considered as a new source of natural compounds? Journal of Enzyme Inhibition and Medicinal Chemistry 31 (1): 754-759. https://doi.org/10.3109/14756366.2015.1063623