



Phytochemical Study and Antibacterial Activity of Crude Alkaloids and Mucilage of *Cordia myxa* in Iraq

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ABSTRACT

The plants of genus *Cordia* comprise of trees and shrubs which are widely distributed in warmer regions. Various compounds like flavonoids, triterpenes, tannins, alkaloids and fatty acids possessing wide range of bioactivities were isolated from different plant parts of *Cordia* species. *Cordia myxa* fruit (family: *Boraginaceae*), is popularly used for the treatment of chest and urinary infections, and as an anthelmintic, diuretic, astringent, demulcent and expectorant agent. The study aims to extract the crude alkaloid and mucilage in the plant *Cordia myxa* under the condition of Iraq's environment and estimation their activity as antibacterial. A wide range of separation techniques were used, based on temperature. A number of solvents were used including ethanol, chloroform and water. These bioactive constituents associated with antimicrobial activity of alkaloid which extracted from leaves and mucilage from fruits. It has been revealed by well diffusion method against pathogenic microorganism such as *Escherichia coli* and *Klebsiella pneumoniae* from urine and *S. pyogenes*, *α hemolytic Strep.*, *Hemophilus influenzae* from sputum. Present study deals with the identification of phytochemicals and antimicrobial activity of leaves and fruit of *Cordia myxa* L. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, saponins, tannins, Glycoside, steroid and coumarin by using different solvent. Antimicrobial activity of alkaloid which extracted from leaves and mucilage from fruits was done by well diffusion method. Mucilage extract showed inhibition effect on gram negative bacteria include *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine at concentration 100,500,250,125,63.5 mg/ml respectively, while no effect of alkaloids extracted from leaves against all pathogenic bacteria.

Keywords: *Cordia myxa*, alkaloid, mucilage, solvent, antimicrobial activity.

INTRODUCTION

The plants of genus *Cordia* comprise of trees and shrubs which are widely distributed in warmer regions¹. *Cordia myxa* fruit locally known as "Bumber" a plant belonging to family *Boraginaceae*², about 300 species have been identified worldwide, mostly in warmer regions³.

The tree keeps its leaves for most of the year. These are broad, alternate, ovate elliptical shaped. The inflorescence carries numerous white flowers. Fruits are round to ovoid shaped drupes, about 15–20 mm in diameter, arranged in clusters. Their white-yellow color turns blackish when dry. The pulp, very tough and mucilaginous, is edible and has a sweetish flavor⁴⁻⁶.

The plant parts like fruits, leaves, stem bark, seeds and roots of most species of plants of the genus *Cordia*, especially *Cordia dichotoma*, *C. myxa*, *C. oblique*, *C. verbenacea*, *C. martinicensis*, *C. salicifolia*, *C. spinescens*, *C. latifolia*, *C. ulmifolia*, among others, has long been used in traditional medicine for cicatrizant, astringent, anti-inflammatory, anthelmintic, antimalarial, diuretic, febrifuge, appetite suppressant, cough suppressant and to treat urinary infections, lung diseases and leprosy^{7,8}.

The pharmacological studies carried out with extracts and purified compounds indicates that the plants of *Cordia* species possess analgesic, anti-inflammatory,

antimicrobial, antiviral and antifertility activities. Various compounds like flavonoids, triterpenes, tannins, alkaloids and fatty acids possessing wide range of bioactivities were isolated from different plant parts of *Cordia* species⁹.

Moreover, it has been reported that leaf extracts of certain species of *Cordia* such as *C. myxa*, *C. francisci*, and *C. serratifolia* have significant analgesic, anti-inflammatory, and antiarthritic activities in rats¹⁰.

Cordia myxa fruit is popularly used for the treatment of chest and urinary infections, and as an anthelmintic, diuretic, astringent, demulcent and expectorant agent¹¹.

The anti-inflammatory properties of the *C. myxa* fruit preparation in the treatment of experimental colitis have been demonstrated¹².

In addition in Africa, the fruit pulp is also employed to treat diarrhoea, dysentery, tuberculosis, wounds, ulcers, to calm abscesses and rheumatic pains and as a vermifuge^{4,13}.

In previous study, Antimicrobial activity tests were performed by Agar well diffusion method against many bacterial strains with highest inhibition zones while there is no effect on the fungal¹⁴.

The study aims to extract the crude alkaloid and mucilage in the plant *Cordia myxa* under the condition of Iraq's

environment and estimation their activity as Anti-bacterial.



Figure 1: Fruiting twig of *Cordia myxa*

MATERIALS AND METHODS

Collection of Plant Materials

Leaves were collected from the *Cordia myxa* tree at the Baghdad city. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and rinsed with distilled water. The samples of plant were taken and kept under shade till drying. The plant material was ground in blender and weighed.

Extraction of Alkaloid

100 gram of grounded leaves was taken in Soxhlet apparatus and 150 ml of 90% ethanol was added. Then it was left for 4 hours. After this time filtered and evaporated to obtain crude extract. The latter was acidified to PH=2 by 4N HCL. Chloroform was then added and the aqueous layer was collected. Neutralized with sodium hydroxide (according to PH-meter). Again chloroform was used to affect the separation. The organic solvent was evaporated and alkaloid collected.

Isolation of Mucilage

Cordia myxa fruits (100 g) were powdered for 5 min in a mechanical blender and soaked in distilled water (350 ml) for 24 h. It was boiled for 1 h under reflux with occasional stirring and kept aside for 2 h for the release of mucilage into water. The material was filtered through a muslin bag and hot distilled water (100 ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely.

Equal volume of ethanol was added to the filtrate to precipitate the mucilage and kept it for one day for effective settling. It was filtered and dried completely and weighed^{14,15}.

Preparation of Leaf Extract for Phytochemical Analysis

Aqueous Extract

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract. The extract was separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02).

Ethanol Extraction

Cordia myxa leaves (100 g) were ground into fine powder using a stainless-steel grinder, deep in 100% ethanol (200 mL) for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

Chloroform Extraction

For preparation of chloroform extract ground plant sample (100 g) was added in chloroform and left for overnight at room temperature.

The extracts were separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02).

Phytochemical Analysis

Saponins

Saponins were detected using the froth test. 2.5 ml of the extract was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is observed for gallic tannins and green colour indicates for catecholic tannins.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Flavonoids

4ml of extract solution was treated with 1.5 ml of 50% methanol solution.

The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Terpenoids

4ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.

Coumarins

5 ml of extract and added 0.5 ml 10% NH₄OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

Sterols and Steroids

Sterols and steroids were sought by the reaction of Liebermann. 10 ml of extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride; we added 0.5 ml of the filtrate. Treated with the reagent of Liebermann Burchardt. The appearance at the interphase a ring of blue-green, showed a positive reaction.

Bacterial Isolates

All bacterial isolates used in this study were isolated from (respiratory tract and urine) samples, collected from patients suffered from many clinical sign and symptoms. These samples collected from patients attended AL-Yarmok teaching hospital, then plated on agar media at 37°C for 18-24h. The pathogenic bacteria were diagnosed by microscope examination, culture and biochemical test APi 20 system (Biomerieux, France). Pathogenic bacteria isolates from clinical samples include *E coli*, *Klebsilla pneumonia* and *Staphylococcus aureus* (urine). In addition to *Strep. pyogen*, *α hemolytic Strep.* and *Hemophilus influenzae* (sputum).

Culture Media

Blood, Mac Conkey, Chocolate agar media were prepared according to the instructions of the Hi-media –India company.

Extract Dilutions

Two fold dilutions were prepared from mucilage extract include (1000, 500, 250, 125, 63.5) mg/ ml. In addition, the alkaloid powder was dissolved by D.W and DMSO to made two fold dilution (1000, 500, 250, 125, 63.5) µg/ml, to determined the effect all these dilutions against the pathogenic bacteria, the D.W and DMSO used as negative control.

Antibacterial Activity

Determination the antibacterial effect of mucilage and alkaloid extract of *Cordia myxa* was carried out by well diffusion method. 100 ml of inoculums (10⁵ CFU/ml : Mac –Farland) of each type of bacteria was spread on muller-hinton agar plate and blood agar. Then let the plate to dry and sterile cork borer (6mm) was used to make wells in agar plates. 100µl volume of each dilution (mucilage and alkaloid) submitted in duplicate wells into media. All plate were incubated at (37°C for 18-24h.). Antimicrobial

activity determined by an inhibition zone surrounding the wells, results consider positive if the inhibition zone more than 6mm. The minimum inhibitory concentration (MIC) of mucilage extraction showing clear zone of inhibition which is completely inhibited the growth for 24h was recorded^{16,17}.

RESULTS AND DISCUSSION

The phytochemical analysis was carried out qualitatively using different standard methods in order to establish the secondary metabolites present in the sample. Table 1 showed the percentage of mucilage extract active compound which was higher (35%) than alkaloid extract (2%). Saponins, alkaloids, curamin were positive by using aqueous and ethanol solvent, while glycosides were positive by using aqueous, ethanol and chloroform solvents. No Terpenoids were detected by using any solvents (Table 2).

Table 1: Percentage of active compound for alkaloid and mucilage

Part Used	Active Compound	Percentage
Leaves	Alkaloids	2%
Fruit	Mucilage	35%

Table 2: Qualitative Phytochemical Analysis of the Extracts of *Cordia myxa* Leaf

Tests	Aqueous	Ethanol	Chloroform
Saponins	+	++	-
Tannins	++	-	-
Steroids	-	-	+
Glycosides	+	+	+
Alkaloids	++	+	-
Flavonoids	+	-	-
Terpenoids	-	-	-
Cumarin	++	+	-

- =Not detected; + = Slight coloration; ++ = Deep coloration

According to the table 3 and Figure 2, the mucilage extract from fruit part was effective as antibacterial against gram negative bacteria isolated from urine samples. The inhibition zones were 15,13,13,12,12 mm in extract concentration 1000,500,250,125,63.5 (mg/ml) respectively against *E. coli* isolate while the inhibition zones were 10,10,8 mm in concentration 500,250,125 (mg/ml) respectively against *Klebsilla pneumonia* isolate.

In addition, there was no antibacterial activity against to *Strep. pyogen*, *α hemolytic Strep.*, *Haemophilus influenzae* isolates from sputum samples.

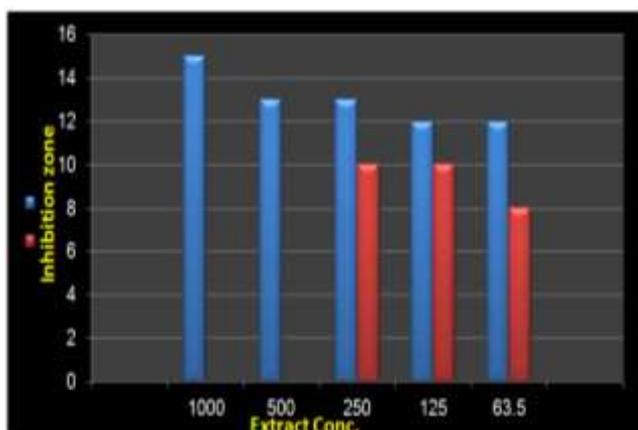
The different concentrations of Alkaloid extract from leaves (1000,500,250,125,63.5µg /ml) were found inactive against all bacterial isolates in this study.

Table 3: Antibacterial activity of *Cordia myxa* mucilage extract

Pathogenic Bacteria	inhibition zones (mm), concentration (mg/ml)					
	1000	500	250	125	63.5	D.W.
<i>E coli</i>	15	13	13	12	12	----
<i>Klebsilla pneumonia</i>	----	----	10	10	8	----
<i>Staphylococcus aureus</i>	----	----	----	----	----	----
<i>Strep. pyogen</i>	----	----	----	----	----	----
<i>α hemolytic Strep.</i>	----	----	----	----	----	----
<i>Hemophilus influenzae</i>	----	----	----	----	----	----

Table 4: Antibacterial activity of *Cordia myxa* alkaloid powder extract dissolved in D.W. and DMSO

Pathogenic Bacteria	inhibition zones (mm), concentration (µg/ml)					
	1000	500	250	125	63.5	D.W., DMSO
<i>E coli</i>	----	----	----	----	----	----
<i>Klebsilla pneumonia</i>	----	----	----	----	----	----
<i>Staphylococcus aureus</i>	----	----	----	----	----	----
<i>Strep. pyogen</i>	----	----	----	----	----	----
<i>α hemolytic Strep.</i>	----	----	----	----	----	----
<i>Hemophilus influenzae</i>	----	----	----	----	----	----

**Figure 2:** Antibacterial activity of *Cordia myxa* mucilage extract against *E. coli* (blue) & *Klebsilla pneumonia* (red)

In the current study, preliminary phytochemical study of *Cordia myxa* was carried out for detection many components like Alkaloids, Flavonoids, Saponins, Tannins etc. *C. myxa* showed positive result for these components by using several solvent but there is negative result for Terpenoids.

This recent study not in line with Pandey *et al.*, who reported that *Cordia myxa* give highest inhibition zone in case of gram positive bacteria (*S. aureus*) and then gram negative bacteria (*E. coli*) in high concentration of leaf extract begin with 4.5%-20%.

Also they cited that case of fungal strains it showed negative results¹⁸.

Kaushik studied the antimicrobial activity of *Cordia myxa*.

According to them results this plant showed good potency in terms of inhibition zones against all the tested bacterial strains. Gram positive bacteria were found to be more sensitive to the *Cordia myxa* extracts¹⁹.

In current work the results showed negative extract activity against all kind of gram positive bacteria including *S. aureus* and good inhibition activity against gram negative bacteria including *E. coli* and *Klebsilla pneumonia* may because we use different part extraction (mucilage), in spite of using alkaloid extract from leaves in this study but it didn't give any positive activity against all strains.

Many studies cited that *S. aureus* multidrug resist because it has plasmid responsible for this multiresistance which increase with the age and physical contact with infected person in community or acquire in hospitals²⁰⁻²².

Different geographical regions are the important reason to different active component of plant lead to different result from region to another.

CONCLUSION

The presence of important active compounds of plant *Cordia myxa* in Iraq region needs more studies to determinate, isolate and measure it as a source for natural health products.

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