

Investigation of Anti-Fungal, Anti-Inflammatory Activity of Cordia Myxa and Screening of Its Functional Groups using Fourier Transform Infrared Spectroscopy Method

Zahraa A. Al-Ajeeli^{1*}, Sarah R. Saleh², Taisir Ali O², Noor Adnan Neema²

¹Al-Qasim Green University, College of Food Science, Dairy Technology Department, Iraq

²AL-Qasim Green University, Faculty of Biotechnology, Department of Medical Biotechnology, Iraq

*Corresponding Author: Zahraa A. Al-Ajeeli

Al-Qasim Green University, College of Food Science, Dairy Technology Department, Iraq

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Abstract: Cordia myxa L is one of the medicinal plants that are applicable in the treatment of many diseases. In many studies, the anti-inflammatory, antifungal, properties of C. myxa have been proven. This study was concerned with the active components and therapeutic effects of C. myxa. Results indicated the presence of the following functional groups alkenes, alkyl halides, aromatics (873.75, 956.69, 1018.41, and 1417.68). The antifungal action of ethyl acetate, ethanol of Cordia myxa extract, fluconazole (FCZ) (standard), and amphotericin B (AmB) (standard) against three fungi and yeast was assessed at the same time (17.08±0.40, 26.00±0.50, 32.59±0.59, and 28.12±0.57) and (25.00±0.46). The activity of the metabolites of Cordia myxa was very high (26.00±0.50) against Cladosporium herbarum. To determine the impact of the oral administration of Cordia myxa extract on the serum enzymes, experimental testing was done in vitro on laboratory rats. Their findings demonstrated that (75.00±3.94, 88.09±4.57, and 24.06±2.03) of Cordia myxa methanol extract, and (121.09±7.01, 150.00±8.37, and 40.00±2.09) of using Di-(2-ethylhexyl)-phthalate, and (43.00±3.06, 60.51±2.68 and 20.94±1.73).

Keywords: Anti-Fungal, Anti-Inflammatory, Anti-Oxidant, Cordia Myxa, FTIR.

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INTRODUCTION

Medicinal plants in addition to aiding in the formulation of most pharmaceutical drugs offer access to cheaper and easily available forms of therapy particularly in areas where modern medicine is unavailable [1]. Cordia myxa fruit (CMF) fruits of the flowering plant belonging to the family of Boraginaceae and is common in tropical and subtropical regions of Asia, Australia and Africa. CMF tree is referred to as Bumber in Iraq. It has anti-oxidant and antibacterial activity in vitro, pharmacological and medical applications. CMF extract has been shown to have antibacterial effect on a host of fungal species, Gram-positive or Gram-negative bacteria. Alyamani *et al.*, made an antibacterial wound dressing using CMF extract as an antimicrobial agent integrated into (PCL/CH) nanofibers. The substance was shown to have significant

antibacterial activity [2, 3]. The outcomes of the present research revealed that the extract of Cordia myxa possessed antifungal effect on three types of fungi and yeast, and that the effect increased with the concentration of the extract and with the time of immersion. The antibacterial activity is mainly caused by phenolic chemicals especially flavonoids [4]. The three areas of action that the bioactive chemicals in Cordia myxa are known to have on bacteria include the alteration of proteins on the bacterial cell surface, inhibition of fungal adhesion, and inhibition of bacterial enzymes. GC-MS showed the presence of significant amount of 19 major compounds with 19 being 0-alpha D-glucopyranosyl- (13) -b -D-fructofuranosyl and 0-alpha D-glucopyranosyl- (13) -b -D-fructofuranosyl [5-7], had been previously implicated as antimicrobial. According to the surface roughness analysis, the statistically significant increase in the roughness ($p > 0.05$) was not

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found after immersion into solutions at different concentrations and durations. These antibiotic effectiveness studies on the different bacterial species confirm the fact that perhaps different types of bioactive chemicals present in *Cordia myxa* leaves have varied activity with a number of target pathogens. This fact supported our conclusion regarding the assortment of *Cordia myxa* ethanolic extracts that were used as a blend of other alternative therapies to treat the infections caused by antibiotic-resistant bacteria. The trends in the resistance of antibiotics that have been detected determine the requirement of careful utilization of antibiotics in clinical use and research studies and the need to conduct more monitoring of these drugs. The bioactive principle that is contained in *Cordia myxa* leaves and is responsible of their antibacterial action needs to be isolated and identified through an in-depth investigation. Thus, a study of their mechanism of action and possible therapeutic applications can become a new step to resolve the issue of antibiotic resistance. To sum up, the total phenolic and flavonoid content of the ethanolic extract of *Cordia myxa* leaves was high, and this is correlated with good antioxidant activity. In this study, this feature was validated by a low value of IC₅₀ in the DPPH assay. As such fungal isolates are even more resistant to certain popular antibiotics, a good alternative could be an ethanolic extract of *Cordia myxa* leaves. To develop natural therapeutic agents, additional studies are recommended in the field of target bioactive molecule extraction and purification, knowledge of mechanism of action and clinical measurements [8, 9]. The aims of our investigation were to evaluate the antibacterial effect of *Cordia myxa* L. extract and report the growth of *Trichophyllum rubrum*, *Cladosporium herbarum*, and *Candida krusei* growth.

MATERIALS AND METHODS

Solution, Identification and Extraction Preparation

The fresh leaves of the *Cordia myxa* tree were taken in the Babylon Province of Iraq. The mature tree was selected and the healthy leaves which were fully mature were collected. The leaves of the tree were then packed into sterile and dehydrated bags of plastic in order to avoid contamination in the lab. When the leaves came, to remove any dust and other extraneous material. The leaves were washed and dried in the shade after seven days to maintain the phytochemical properties. They are also dried and then grained into powder using an electric grinder. The ethanolic extract was prepared by keeping 250 mL of 96% ethanol and 15 g of powdered defatted *Cordia myxa* leaves in a Soxhlet apparatus between 2 and 8 °C to prevent the moisture absorption as well as microbiological contamination. Eight hours were used in the extraction process. The evaporation of the solvent following the use of a rotary evaporator was done at a temperature of 450°C, to yield the dried ethanolic extract. The extract was placed in impermeable containers at 2 to 8 °C.

Exploring the Antifungal Property of Crude Compound Extracts

The antifungal properties of crude extracts at the concentration of 300 mg/mL were evaluated using the mixing method with the Sabouraud dextrose agar (SDA). 0.1 ml of each of the concentrations was transferred to a Petry dish. A 5 mL disc of each fungus was taken using the sterile cork borer and was placed on the culture media surface after the SDA medium was poured onto it and the dishes were left to solidify. The petry dishes are incubated at 25 °C +/- 2, 7days. The antifungal properties of the extract is determined by measuring the diameter of the inhibitory zone in millimeters (mm) using a ruler.

Cordia Myxa Analysis, Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the two native and defatted GLVs were recorded by the data of an FTIR instrument that was driven and processed with PC-based software. A small portion of powdered samples was prepared in KBr to form pellets and a thin layer was formed by the pressure application to get prepared before FTIR analysis. The data concerning the infrared light transmittance was collected within a range of wave numbers of 4000 cm⁻¹ to 500cm⁻¹. Each sample was subjected to three distinct analyses, which were used as control (untreated KBr pellets). The spectral data were related to a reference and the functional groups present in the sample were determined.

Drug Administration and Experimental Animals

Male albino rats of good healthy conditions with a weight of 200 to 250 grams were the experimental animals. The atmosphere was kept at a constant temperature, and they were kept in cages consisting of polypropylene and fed typical laboratory food. The rats had to be acclimatized to the facility before they were involved in the experiments which took at least eight hours. The total number of the rats was twelve divided into three groups of four. Group I animals were used as a standard control, and their controls were maize oil, which was used as a vehicle control and 100 mg/kg Di-(2-ethylhexyl) phthalate were the positive controls in each experiment that involved the use of methanol fraction of *Cordia myxa*. Group 1 was stocked with 100 mg/kg of Di-(2-ethylhexyl) phthalate and Group 2 and 3 were stocked with *Cordia myxa* fractions.

Analysis of Statistics

The results of all the biological research were expressed using the means + S. E.M. The test and (ANOVA) were applied by Dunnett to achieve the significance in relation to the control group. Statistical significance was specified as p value values below 0.05, 0.01, and 0.001 (p<0.05, p<0.01 and p<0.001). In calculation of statistics, Graph Pad Prism 5 was used.

RESULTS AND DISCUSSION

FTIR is a physicochemical analytical technique to provide an accurate picture of the metabolic composition of the leaves at a given time. It is possible to find out even the slightest alterations in both primary and secondary metabolites of the leaves with the help of the IR spectra [12, 13]. FTIR is employed in the determination of the intensity of absorption spectra attached to the molecular composition or existence of a particular chemical functional group and clarification of the structure of unknown composition. FTIR has been used to identify plant and fungal species along with the

determination of the metabolites in plants [15-17]. The encouragement of the FTIR method to determine the spectral variation of the targeted compounds of GLVs would influence the processing of the results after harvest. This was the first study to be done on the application of the FTIR method to establish the variation of the antioxidant activity of native and defatted foliage of green vegetables *F. maxima* in particular. Nevertheless, these GLVs do not have any similar publications. Alkenes, alkyl halides, and aromatics (873.75, 956.69, 1018.41, and 1417.68) were identified as the following functional groups.

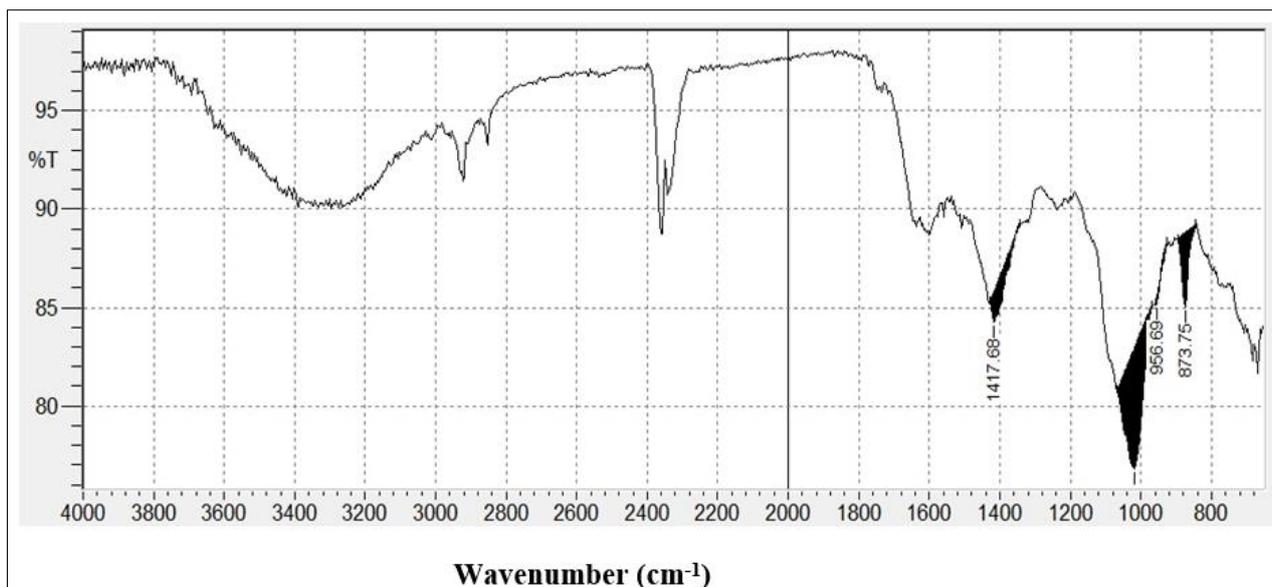


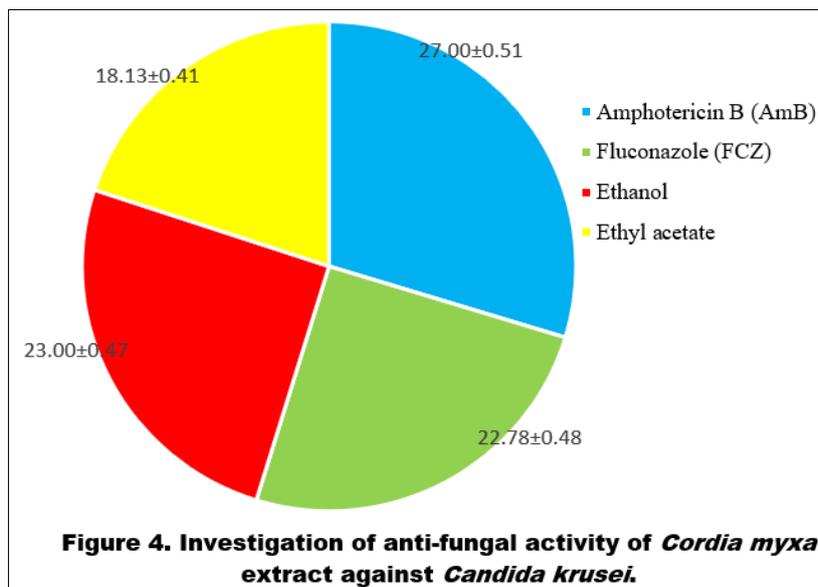
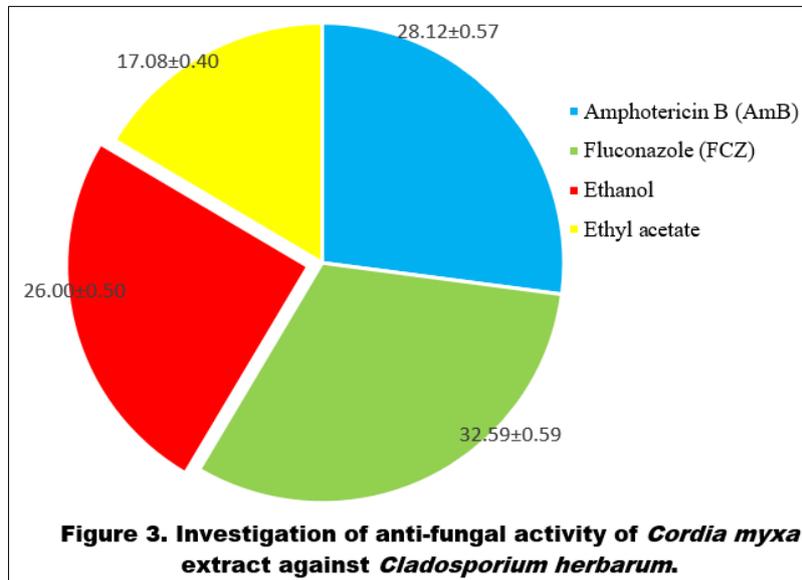
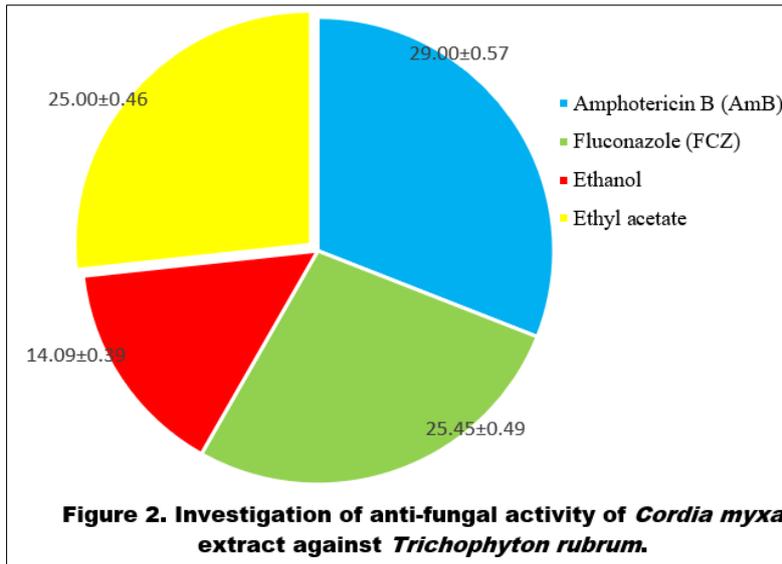
Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Cordia myxa*.

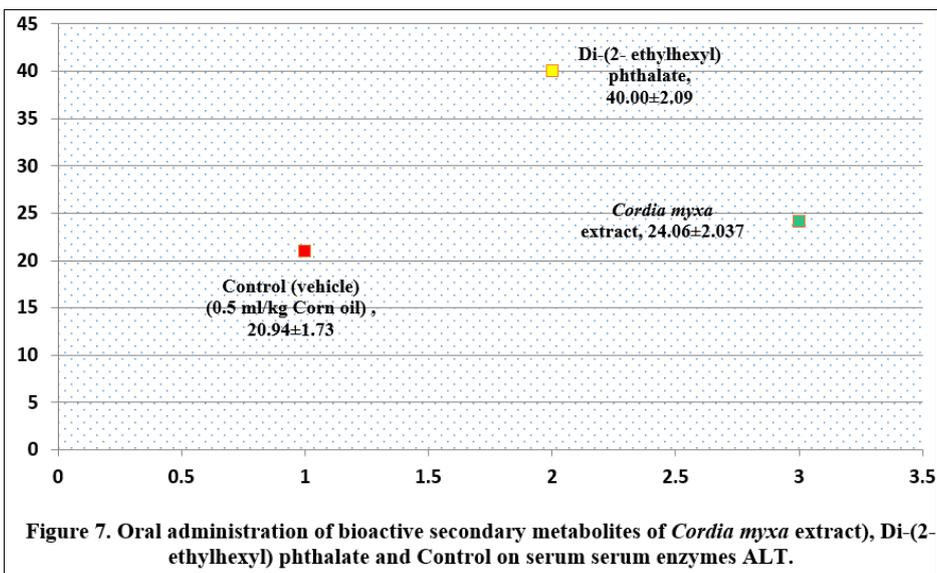
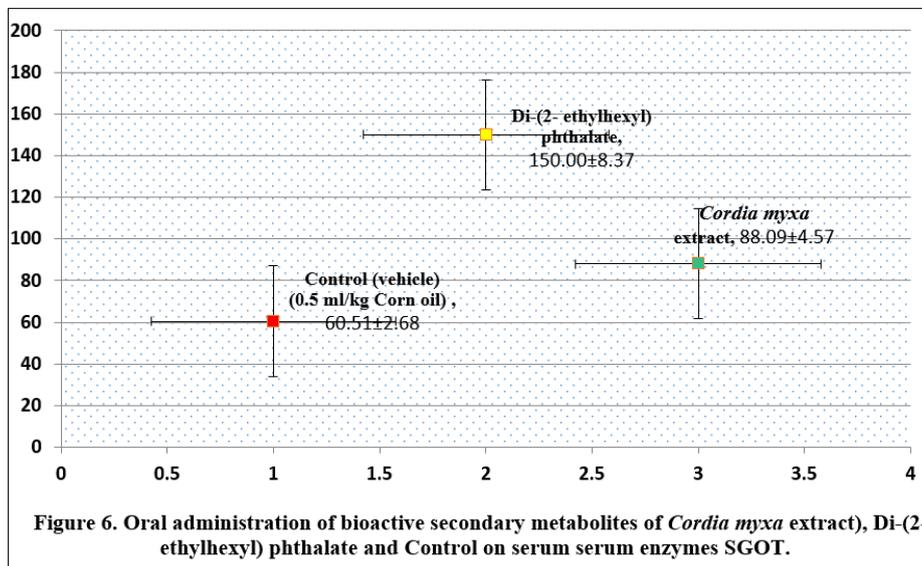
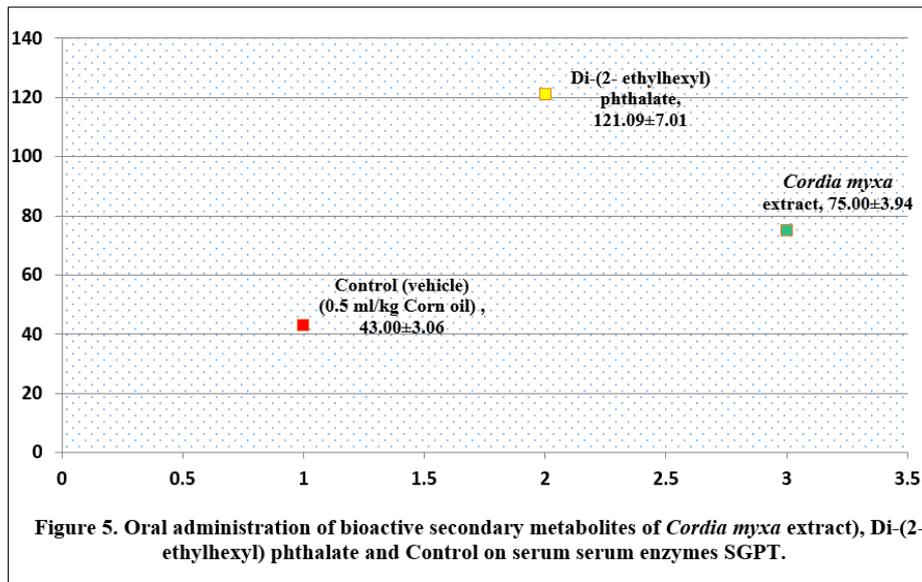
Table 1. FT-IR peak values of solid analysis of *Cordia myxa*.

No.	Peak (Wave number cm ⁻¹)	Intensity	Corr. Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	873.75	85.048	3.776	Strong	=C-H	Bending	Alkenes	650-1000
2.	956.69	85.168	0.255	Strong	=C-H	Bending	Alkenes	650-1000
3.	1018.41	76.824	6.157	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1417.68	84.284	1.477	Medium	C=C	Stretch	Aromatic	1400-1600

Ethyl acetate, ethanol of *Cordia myxa* extract, fluconazole (FCZ) (standard), and amphotericin B (AmB) (standard) were examined regarding their antifungal properties against three fungi and yeast. recorded against *Cladosporium herbarum* (17.08±0.40, 26.00±0.50, 32.59±0.59, and 28.12±0.57) and *Trichophyllum rubrum* (25 The activity of the metabolites of *Cordia myxa* was very high (26.00±0.50) with *Cladosporium herbarum*. Laboratory rats were used in in

vitro experiment tests in order to determine the impact of oral administration of *Cordia myxa* extract on the serum enzymes (SGPT, SGOT and ALP). The findings indicated that the *Cordia myxa* ethanol extract affected the enzymes (75.00±3.94, 88.09±4.57 and 24.06±2.03) and Di-(2-ethylhexyl) phthalate affected the enzymes (121.09±7.01, 150.00±8.37 and 40.00±2.03) and Control.





The ethanol extract had the most anti-inflammatory properties when compared with other fractions. This effect may possibly be caused by its high levels of flavonoids and other phenolic chemicals including phenyl propanoids. The dichloromethane fraction and the entire extract of ethanol exhibited the best anti-inflammatory properties compared to the indomethacin. The anti-inflammatory effect of flavonoids was demonstrated in many ways, such as the inhibition of the cyclooxygenase and 5-lipoxygenase system, the blockage of eicosanoid synthesis, and neutrophil breakdown inhibition [18-21]. Their potent antipyretic effect could be explained by the presence of flavonoids and sterols in the entire extract of *Cordia myxa* L. and different fractions. Their analgesic effect may be due to sterols which have been reported to have analgesic effects [22, 23]. The final but not the least, the hypoglycemic effect is also in line with the published studies on the whole ethanol extract which showed that the hypoglycemic effect is through the inhibition of the alpha-glucosidase enzyme. Consequently, it can reduce the level of glucose or inhibit glucose absorption. It is a known fact that polyphenols, alkaloids, and flavonoids of the plants have a strong anti-inflammatory effect. Fruit cordia myxa is rich in polyphenolics and this maybe the reason it prevents the injury of rat liver [24-27]. Since *Cordia dichotoma* frost preparations have tannins, flavonoids, alkaloids, glycosides, saponins, as well as carbohydrates, it is assumed that it has anti-inflammatory effect on paw edema in rat caused by carrageenan and dextran. *Cordia dichotoma* has also been shown to be useful in the rate models of wound healing and stomach ulcers because of its fruit. The analgesic and anti-inflammatory effects of these medicinal plants were tested in rats by the use of leaf extract of the five *Cordia* species. The anti-inflammatory effects of *Cordia myxa* fruit in a colitis model, demonstrated that a preparation of sepestan lowers the concentration of oxidant stress factors which lead to colitis development, increasing the total antioxidant status level and returning it to the normal range [28]. Moreover, *Cordia myxa* is also a good source of several nutrients as far as their bioavailability is concerned. They suggested that *Cordia myxa* fruit could have anti-inflammatory effect partly because of its antioxidant effects and because of the replenishment of the trace elements deficiency in the inflammatory liver, colon, and plasma. More recent studies have demonstrated that the pro-inflammatory mediators p38 and JNK3 were the most inhibited by the rosmarinic acid and rosmarinic acid ethyl ester of the ethanolic extract of *Cordia americana* [29, 30]. Further studies are required to identify the specific chemical action of the object and the exact constituents responsible to this action.

CONCLUSIONS

Due to the presence of a large number of phenolic compounds, flavonoid, and mucilage, *Cordia myxa* is a traditional medical plant that is highly anti-inflammatory. One of its actions is that it suppresses COX-2 and possesses anti-oxidant properties to assist in

curbing inflammation in diseases such as colitis and respiratory diseases. Evidence has shown that its extracts are capable of lowering inflammation in animal test subjects and this could open the way to the development of newer anti-inflammatory drugs. To treat inflammatory diseases, *Cordia myxa* fruit crude extract can be regarded as an alternative approach of anti-inflammatory treatment or supplementary therapy that allows patients to take fewer doses of conventional anti-inflammatory drugs and decreases the adverse outcomes of conventional medications.

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