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Original Article

Investigation of Anti-Bacterial, Antioxidant (Peroxynitrite scavenging and Hydroxyl radical scavenging) Activity of Cordia myxa and Screening of Its Functional Groups Using Fourier Transform Infrared Spectroscopy Technique

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Abstracts:

There has been a lot of recent buzz about medicinal plants as a viable alternative to chemical treatments due to their inexpensive production costs and relative lack of adverse effects. However, phenolics and flavonoids derived from plants have many positive biological effects, including antioxidant, anti-inflammatory, and antibacterial actions; these compounds are very lucrative for the cosmetics, pharmaceutical, and food industries. Reactive oxygen species (ROS) and free radicals are harmful molecules that can be produced by cells in the human body. These molecules can harm live cells and lead to various clinical disorders. Discovering the bioactive components and antibacterial activities of Cordia myxa fractions is the main objective of the current work. The fruits that were picked were thoroughly rinsed with a combination of tap water and deionized water. The crop was harvested when it was still edible, then dried in the shade and pulverised into a powder. In preparation for the following tests, the fruit extract was diluted in an appropriate proportion of ethanol. Subsequent to extraction, all tests were carried out no later than 72 hours. As part of the preparation for FTIR analysis, Peak (Wave number cm-^{l}), Peak (Wave number cm-^{l}), were 675.09, 692.44, 738.74, 813.96, 974.05, 1008.77, 1049.28, 1093.64, 1232.51, 1276.88, 2922.16, 1276.88, 1606.70, 1647.21. Streptococcus pyogenes was significantly inhibited by the Cordia myxa metabolites (28.94 ±0.46).

Keywords: Cordia myxa, antibacterial, Functional Groups, FTIR Technique

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Introduction:

This plant is locally known as Bumber and the scientific name of this species is Cordia myxa L., it is widely used in curing illnesses that affect the urinary tracts and also cough related ailments associated with chest complications. Various functions include as a liver tonic; it has anti diarrhea, demulcent, diuretic and anti gastritic properties as well as being anthelmintic. Cordia species has afforded many formulations in the traditional medicine practice for the management of osteoarticular ailments. In this regard, analysis of literature reveals that five species of cinnamon include; C. Specifically, the five identified species including Chrysobalanus icaco, Coumarouna francisci, Calophyllum martinicensis, Calophyllum myxa, C. serratifolia, and C. ulmifolia work analgesically, anti-inflammatory, and antiarthritic. Inside plants there has evolved the capacity to generate new bioactive secondary metabolites, and plants in general encompass a broad range of activities that are of medical utility. The potential of these metabolites is not only based on their ability to treat diseases but in its extraction, they offer a probable resource for creating new drugs, and improvements to food's flavour and additional uses such as food preservatives and other commercial products. The plants contain a wide range of secondary metabolites some of which are the phenolics and flavonoids. For instance, these chemicals are produced through normal body processes, and other exogenous factors that impact on the overall body functioning. However, phenolics and flavonoids also may be useful to man as they help to decrease the potentiality of degenerative /chronic diseases that affect the plants. Nevertheless, the identified plant-originated phenolics and flavonoids exhibit multiple Vorbidden Person positive biological impacts such as antioxidant, anti-inflammatory, and antibacterial characteristics [1-3]; these compounds are excessively profitable for the cosmetic, pharmaceutic, and food market sectors. Free radicals are toxic chemical species that encompass reactive oxygen species too in this case. They are toxic to live cells and cause clinical diseases on human body. Most natural and synthetic antioxidants have proved to quench free radicals, hence reduce the occurrence of this adverse health condition However there are some negative impacts that are as a result of use of synthetic antioxidants [4, 5]. Through advancement in technology such as nanobiotechnology, natural products derived from medicinal plants can turn out to be authoritative cure to germs which ail people. Hence, it is essential that new pharmaceuticals from organic sources are developed to help mankind. Falling under the Boraginaceae family, Cordia myxa is a flowering plant that is found in areas that experience sub tropical to tropical climate such as Africa, Australia and the Asia. In a recent carried out study, Cordia species has been found that the fruits of these species are including source of protein, carbohydrate, essential fatty acids, bone, and minerals [6]. The objective of this study is to determine the functional groups and ethanolic and other fractions of the extract of Cordia myxa fruits that possess antibacterial properties by applying the FTIR technique.

Materials and Methods

Collecting plant materials

All the analytical-grade chemicals in the study were purchased from Sigma-Aldrich Chemical Co based in St. Louis, MO, USA. The fruits were collected from the Cordia myxa, tree found specially in Hillah City of Iraq. Subsequent to washing with water and physically examination, fruits that were deemed to be either physically or microbiologically spoiled were sorted out.

Getting the extract ready

The fruits that were picked were well washed using tap water and pure water which was de-ionized. The crop was chewed when it was still fresh and then sun-dried to obtain a powder. Subsequently, a conical flask was used to measure 100 g of powder into the 250ml of ethanol. A magnetic stirrer was then applied to stir the mixture at room temperature for 3 hours of which the condition was that the flask had to be covered to ensure that it was dark. Last, the mixture was filtered with the help of Whatman No. 1 Filter paper. This procedure was followed by another one after which the filter paper was transfered to a conical flask. The extract was then collected and till the solvent was completely evaporated it was allowed to dry in a rotavapor at a temperature of about 65^0 C [7-9]. The cumulative weight of the resultant crude extract was measured and stored at 4 °C for the percentage yield assessment. The solvent used in the extraction process was a 70% ethanol, and 30% water solution was used. Before the following tests, the fruit extract was diluted in ethanol in the correct proportion. All tests in regard to the sample were conducted not later than 72 hours after extraction.

Analysis of Cordia myxa through the Fourier transform infrared spectroscopy (FTIR)

These FTIR spectra for each GLV were derived through the experimental work of running and processing a broad set of data acquired from an FTIR instrument, mainly applying PC-based software. This we did so that we could obtain the FTIR spectra of these two types of GLVs. Sample preparation for FTIR analysis was carried out in the following manner: A small amount of crushed leaf samples was mixed with KBr and pressed into pellets for analysing the samples while at the same time making the examined mixture thin layer. At the same time, data was collected in the wave number range of 4000 cm1 to 500 cm1 as it was important to obtain credible and academic information on the transmission of Infrared light. In the present case, all the experimental samples were subjected to three distinctly different analyses with untreated KBr pellets being used as negative control.

Detecting the Minimum Inhibitory Concentration (MIC) of the compound Optimal antimicrobial activity against microorganisms and bacterial strains

The experimental screening has been already several types using ordinary pathogenic bacteria; for example, Streptococcus pyogenes, Streptococcus agalactiae, Escherichia Coli and Bacillus Cereus. As a matter of fact, they were incubated at 37 degrees celcius for 24 hours in a petri dish on 10ml of experimentally aseptic nutrient brothy. The incubation period for all the plates was only 24 hours with 37°C as the optimal temperature. An optical microscope was used in the experiment to establish the shape of the bacterium. This marks the turbidity of the 0. 5 McFarland standard, which was prepared fresh in the laboratory on Mueller-Hinton agar and was equal to 1. 5 x 10 to the power 8 colony-forming units per millilitre, was diluted in the laboratory to bring the number of colonies in each strain to the observed level. The sterile saline was then utilised as the control.

Peroxynitrite and hydroxyl radical inhibitory/binding ability

"Scavenging peroxynitrite"

Using an upper pre-frost precipitate an absorbance of 302 nm was recorded for the peroxynitrite solution, which gave a molecular extinction coefficient of 1670 M-1 cm-1. The measurement of peroxynitrite scavenging activity was done using an Evans Blue bleaching assay. Minor changes were made to the process performed in the test that corresponds to the standard. Concentration of peroxynitrite: 1 mM, EB: 5 μmol/L; and different volumes of plant extract 0-200 μl. After 30 min of incubation at 25 ° C, we took a measuring at the wave length 611 nm. These conclusions were based on the assessment of ONOO-% scavenging of the control and sample groups. Trial runs of the model are less than 6 runs. The reference material selected for this endeavour was gallic acid.

Scavenging hydroxyl radicals

It is also notable that it was possible to obtain hydroxyl radicals with the help of the system based on Fe3+, ascorbate, EDTA, and H2O2 – Fenton reaction. The final volume that contained the mixture was 1 millilitre. EY One fifth of the reaction mixture was poured into 1 ml of 2. 8% TCA alteration fixed following the incubation process of one hour at 37˚C. It is then processed by innoculation to 90C for the purpose of obtaining colour for fifteen minutes. A portion of the solution was allowed to cool and the absorbance at were read at 532 nm with a suitable blank. Every test was conducted six times. To compare the results we used mannitol, which is a classical OH scavenger, as a control. In order to calculate the % inhibition, the sample solution to the blank solution was made.

Quantitative Evaluation

Statistical evaluation. Its analysis we made using the software called SPSS, which is short for “Statistical Package for the Social Sciences”. The average of three individual counts plus or minus the standard deviation of all the three measurements and p value derived from the ANOVA test. Depending on the study, the p-value has to be lower than 0.05 to be considered as statistically significant at the realistic level of analyzing the given data.

Results and Discussion

A fast and powerful method for examining cell wall components and putative cross-links, Fourier Transform InfraRed (FTIR) spectroscopy may non-destructively identify functional groups and polymers and give a wealth of information regarding their in vitro organisation. Cell wall alterations in muro can be tracked using FTIR spectroscopy, which can be triggered by a variety of reasons such growth and development, mutations, or biotic and abiotic stressors. The

massive amounts of data collected and the presence of overlapping band components initially restricted the use of FTIR technology [10, 11]. The interest in and use of these spectroscopic methods, however, have grown in recent years thanks to the proliferation of statistical tools and the improvement of computer systems that process the spectra. Peak (Wave number cm-^{l}), were 675.09, 692.44, 738.74, 813.96, 974.05, 1008.77, 1049.28, 1093.64, 1232.51, 1276.88, 2922.16, 1276.88, 1606.70, 1647.21. Alcohols bound to micromolecules, free alcohols, alkanes, aromatic compounds, imines, oximes, ketone or alkene, and phenol were all detected in the laboratory analysis using FTIR. The acquired data made it quite evident that the bands in the infrared region are mostly caused by the chlorophyll molecule. This is mainly due to the fact that other components are concealed by the chlorophyll molecule. Artemisia annua leaf extracts were examined in vitro for their antibacterial efficacy against four distinct microbial species. The results for Streptococcus pyogenes were 26.95±0.42, 21.06±0.39, and 28.94 ±0.46 for the methanol, ethyl acetate, and ethanol fractions, respectively. The results for Escherichia coli were 22.02±0.30, 16.08±0.26, and 26.05±0.42. Note the Streptococcus agalactiae strains 25.17±0.37, 20.19±0.25, and 23.27±0.27. The results for Bacillus cereus were 18.16±0.27, 26.17±0.40, and 28.91±0.39, compared to 19.00±0.32 for Rifambin and 26.41±0.36 for Bacteracin. Streptococcus pyogenes was significantly inhibited by the Cordia myxa metabolites (28.94 ±0.46).

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

No.	Peak (Wave number cm-^{l})	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	675.09	67.825	1.011	684.73	671.23	2.209	0.033	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	692.44	69.075	1.098	705.95	686.66	3.008	0.076	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	738.74	72.075	2.274	750.31	732.95	2.372	0.151	Strong	=C–H	Bending	Alkenes	650-1000
4.	813.96	76.441	2.172	823.60	804.32	2.118	0.099	Strong	=C–H	Bending	Alkenes	650-1000
5.	974.05	65.287	0.687	975.98	941.26	5.103	0.077	Strong	=C–H	Bending	Alkenes	650-1000
6.	1008.77	54.765	7.948	1039.63	977.91	14.254	1.759	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1049.28	58.347	2.245	1056.99	1041.56	3.494	0.131	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1093.64	64.409	3.812	1126.43	1087.85	6.008	0.401	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1232.51	80.641	0.860	1236.37	1213.23	1.937	0.064	Strong	C-F	Stretch	alkyl halides	1000-1400
10.	1276.88	80.140	2.278	1288.45	1263.37	2.246	0.127	Strong	C-F	Stretch	alkyl halides	1000-1400
11.	1606.70	79.503	3.556	1618.28	1581.63	3.219	0.402	Bending	N-H	Stretch	Amide	1550-1640
12.	1647.21	79.220	4.828	1664.57	1639.49	2.059	0.286	Variable	C=C	Stretch	Alkene	1620–1680

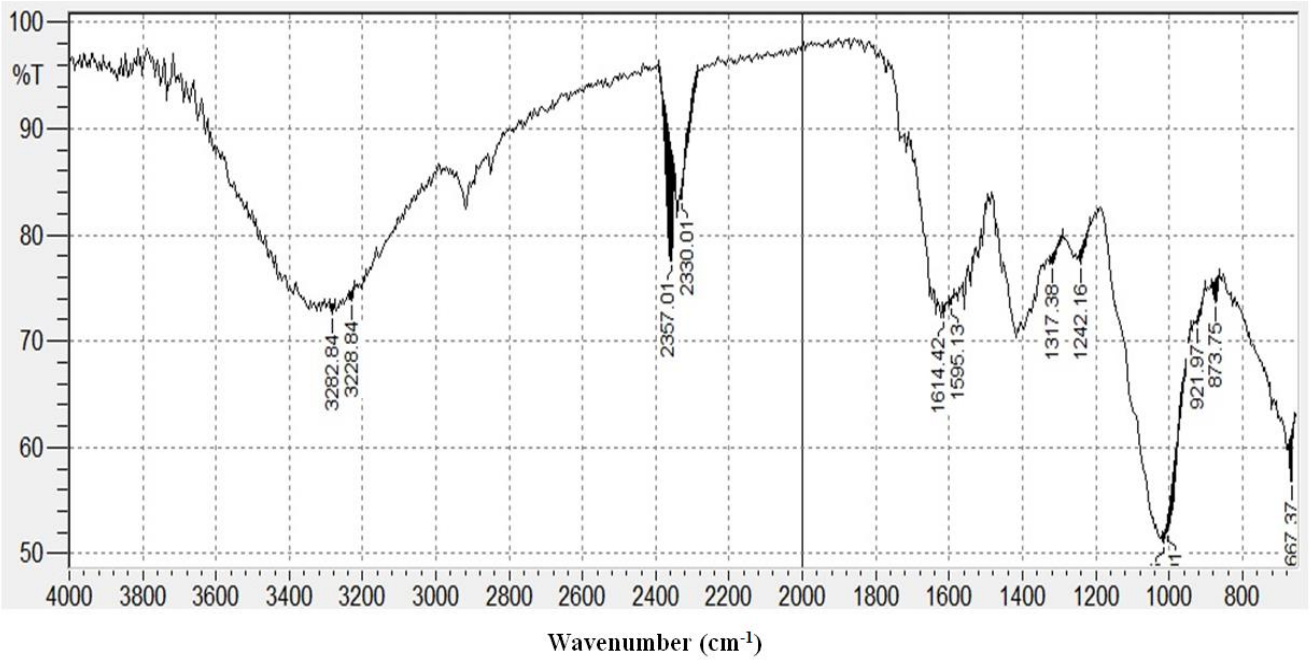

Figure 1. FT-IR peak values of solid analysis of ethanolic fruit extract of *Cordia myxa*.

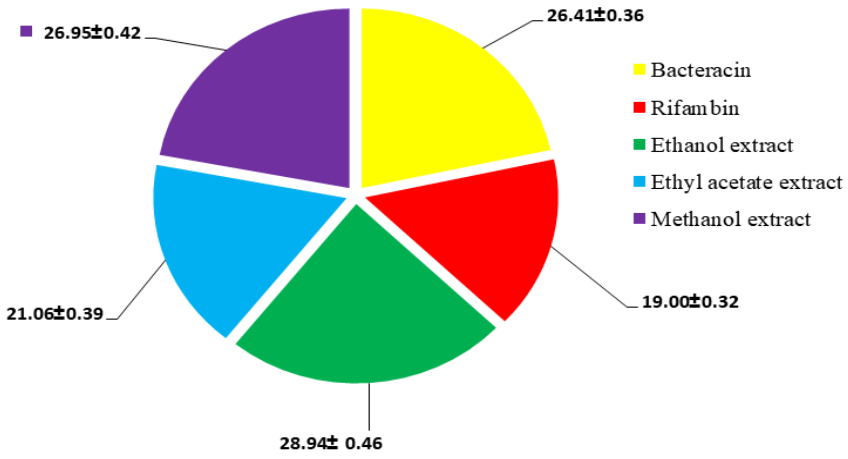

Figure 2. Inhibition Zone (mm) of various bioactive compounds derived from *Cordia myxa* and conventional antibiotics against *Streptococcus pyogenes*

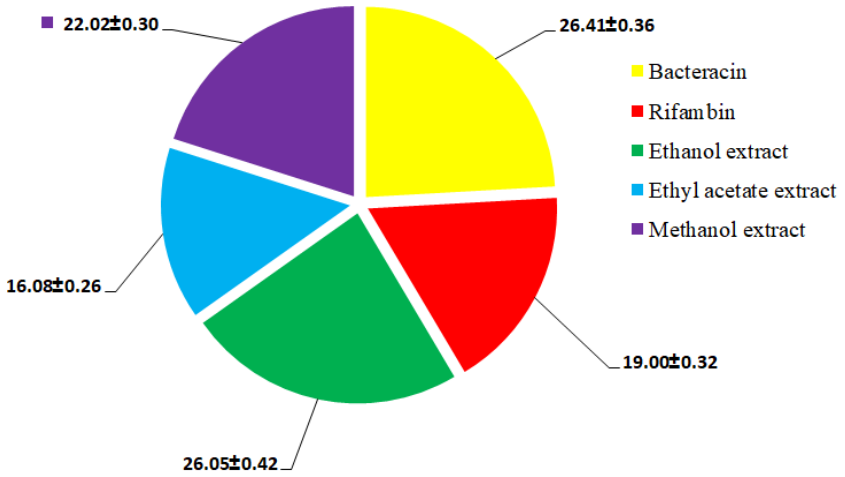

Figure 3. Inhibition Zone (mm) of various bioactive compounds derived from *Cordia myxa* and conventional antibiotics against *Escherichia coli*

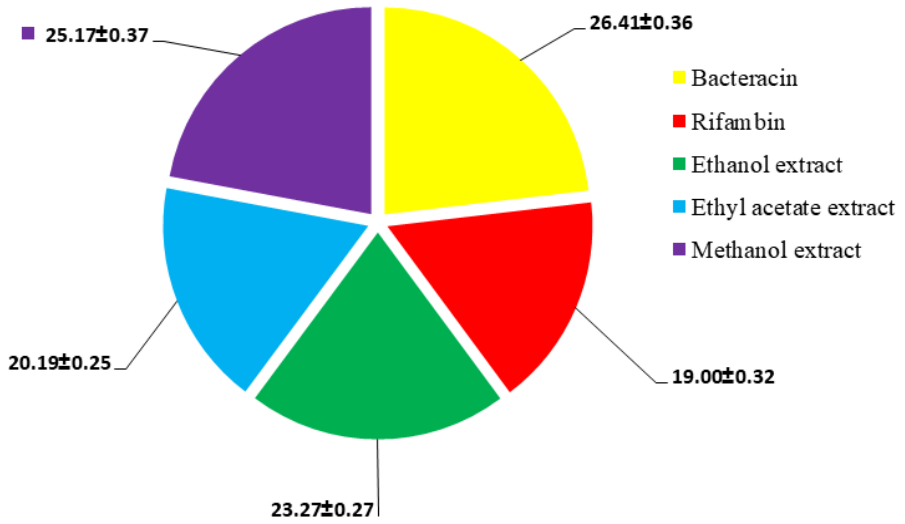

Figure 4. Inhibition Zone (mm) of various bioactive compounds derived from *Cordia myxa* and conventional antibiotics against *Streptococcus agalactiae*

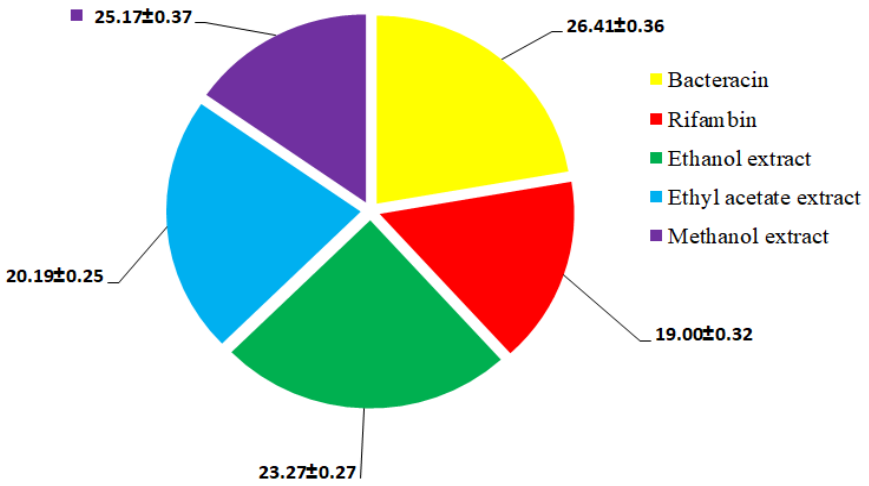

Figure 5. Inhibition Zone (mm) of various bioactive compounds derived from *Cordia myxa* and conventional antibiotics against *Bacillus cereus*

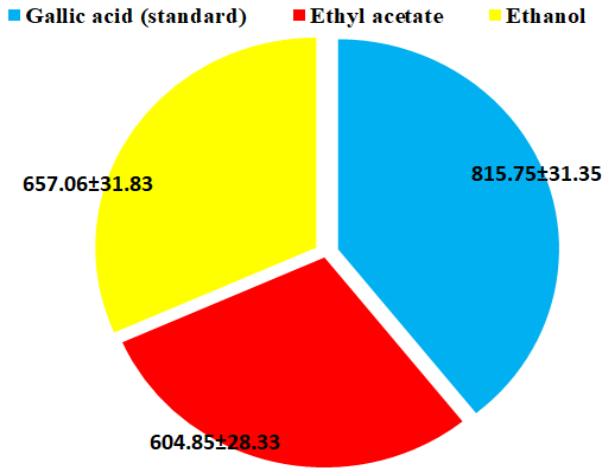

Figure 6. Antioxidant activity (Hydroxyl radical scavenging) of fruit extract (Ethanol fraction Ethyl acetate fraction, and Gallic acid (standard)) of *Cordia myxa*

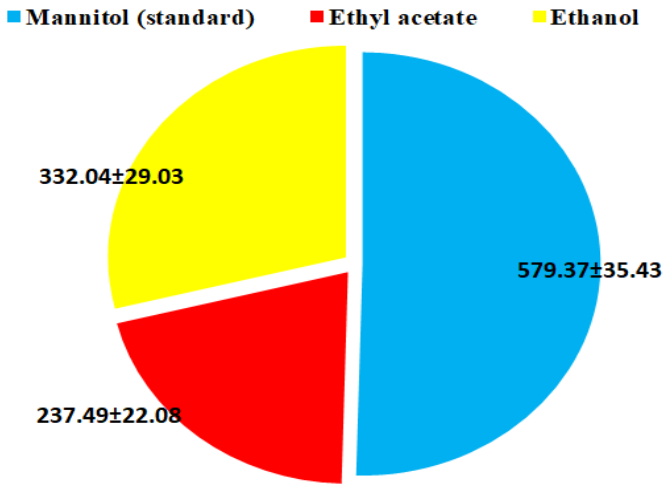

Figure 7. Antioxidant activity (Peroxynitrite scavenging) of fruit extract (Ethanol fraction Ethyl acetate fraction, and Mannitol (standard)) of *Cordia myxa*

Exploring the antioxidant activity of Cordia myxa fruit extract in various solvents, including ethanol, ethyl acetate, and standards, with respect to peroxynitrite and hydroxyl radicals. Figure 6 shows that several types of extracts were recorded, including an ethanol fraction, an ethyl acetate fraction, and a standard, with respective concentrations of 657.06±31.83, 604.85±28.33, and 815.75±31.35 gallic acid (standard). The levels of hydroxyl radical scavenging potential were 332.04±29.03, 237.49±22.08, and 579.37±35.43 for mannitol (standard), respectively. Illustration 7.

Using CMF as an example, this study found that it inhibited the growth of several bacteria, including S. aureus, E. coli, S. enterica, B. subtilis, and P. aeruginosa. This finding is in line with a prior study that found that CMF fruit extracted with ethanol had antibacterial effects against S. aureus and E. coli. According to these findings [12-16], CMF's ethanol extract may be used to prevent the growth of bacteria that cause food poisoning, including S. aureus, E. coli, S. enterica, B. subtilis, and P. aeruginosa. These new findings corroborate those of earlier research on the antimicrobial properties of Cordia plant extracts. Aqueous and alcoholic extracts of Cordia myxa have been shown in earlier research to suppress the growth of Pseudomonas fluorescens, Salmonella, Shigella, and Escherichia coli in a concentration-dependent manner. The biological effect of plant extracts, which can be exerted either singly or in combination [17-20], is attributed to the naturally occurring compounds with chemically complex compositions. Flavonoids are the most famous phenolic chemicals because of their strong antioxidant properties. Among the several mechanisms [21, 22] by which phenolic compounds exert their antibacterial effects include adsorption and disintegration of microbial membranes, ion deprivation, enzyme interaction, and interaction with membrane transporters.

Conclusion

The GC-MS profile analysis of the ethanol extract from the fruit Cordia myxa revealed the presence of several phytochemical components. The crude extract showed antimicrobial effects on different pathogenic microorganisms that may be attributed to the phytochemicals in the CMF extract, as well as high antioxidant activity in vitro by blocking DPPH, in comparison to the standard (ascorbic acid). Streptococcus pyogenes was significantly inhibited by the Cordia myxa metabolites (28.94 ±0.46). Thus, our results suggest that there is a great opportunity to use Artemisia extracts in the development of new treatment regimens for infectious diseases that are resistant to current methods.

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