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To cite this article: Abbas D M Al-Maliki *et al* 2021 *J. Phys.: Conf. Ser.* **2063** 012020

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## Estimation and evaluation of medicinal efficacy of maximum inhibitory concentration of alkaloids extracted from Iraqi *Cordia myxa* leaves against some pathogenic bacteria.

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**Abstract.** *Cordia myxa* is considered as one of traditional medicinal plants abundant in many countries including Iraq . Because of the healthy importance of this plant therefore the current research was established to extract the alkaloids from the its leaves with extraction percentage equal to 5.8% Preliminary qualitative detections were achieved for the alkaloids by using various chemical reagents. The medicinal efficacy of these alkaloids was determined depending on maximum inhibitory concentration (Max IC) which has value equal to 150 mg/ml and this concentration recorded inhibition zone diameters values equal to 30,22,26,25 and 30 mm against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus Sp.* and *klebsiella Sp.* bacteria. So *Cordia myxa* leaves alkaloids can be used as natural chemical drug to treat the most infections and inflammatory caused by these pathogens .

### 1. Introduction

The healthy value and medical importance of various traditional plants belong to existence of many active natural metabolites in the different parts of medicinal plants spreading in the world-wide. The chemical production of these active biochemical compounds takes place through secondary metabolism pathways [1] [2]. Various current studies proved the advantages of the environmental natural plants because of their important sources to produce drugs belonging to nature origin. Excellent therapies were isolated, purified and characterized from variety of medicinal plants depending on their big biochemical efficacy and chemical ability to treat various infections and inflammatory caused by different microorganisms. The necessary benefit of the active metabolites comes from they have no side effects opposite synthetic drugs[3][4]. The healthy and medical



importance of various traditional medicinal plants results from existence of many potent chemical families as secondary metabolites are anabolized biochemically in different parts of these plants according to metabolic pathways catalyzed by required enzymes. The active metabolic compounds are represented by phenolic compounds, glycosides, terpenes, alkaloids, essential oils, flavonoids, coumarins, xanthone, tannins, steroids and Saponin[5][6][7].

Alkaloids are considered as one of active secondary chemical metabolites abundant in various medicinal plants and they are chemically heterocyclic basic nitrogenous compounds have one or more nitrogen ring. Also the molecule of alkaloid has nitrogen atom is attached to at least two carbon atoms. The alkaloidic compounds are derived basically from primary metabolism especially from amino acids. Alkaloids have medicinal and dramatic properties and they were used successfully as antibacterial, antifungal, analgesics, miotics, antileukemic and antifungal agents [8][9].

*Cordia myxa* is considered as one of medicinal traditional plants spreading in different countries in the world. It is a flowering species belongs to Boraginaceae family and the size of this plant is medium has wide leaves and the tree is deciduous. There are common names for *Cordia myxa* such as pidar, lasura, spistan, naruvilli and wanan. Also this plant is used as popular food in various countries and a good substance to produce materials such as potassium, calcium, zinc and iron [10][11]. Various studies were achieved to examine the chemical content of *Cordia myxa* parts especially fruits where glycosides, gums, tannins, alkaloids, coumarins, flavonoids and phenolics were tested qualitatively. Also the therapeutic importance of the fruits as anti-inflammatory and antiparasitic were evaluated [12][13]. The chemical constituents in fractions of *Cordia oblique* in methanolic extract of leaves were estimated and determined by gas chromatography- mass spectroscopy technique. A research indicated presence of sesquiterpene, amino acids, azafirin, oleic acid, phthalic acid, steroids, morphinan and galactopyranoside [14]. Also alkaloids of *Cordia myxa* leaves were extracted and they were examined on survival of mustard aphid [15].

The present research was done for estimation, investigation and evaluation the biochemical and medicinal efficacy of alkaloids extracted from *Cardio myxa* leaves at maximum inhibitory concentration against growth of five pathogenic bacteria.

## 2. Materials and Methods

### 2.1 Preparation of plant

The Leaves belonging to *Cordia myxa* were collected from Al-Qurna district at Basrah Governorate in Iraq. The plant was taxonomied, cleaned with distilled water, dried, powdered and kept in a dark glass container for the sake of application of various analysis.

### 2.2 Extraction of alkaloids from *Cordia myxa*

Twenty five grams of *Cordia myxa* leaves powder were added into 250 ml of ethanolic-acetic acid (10% v/v) and the mixture was stirred by using magnetic stirrer for eight hours. Then the contents were filtered and the precipitate was removed. The filtrate was concentrated to quarter of its volume then 5 ml of concentrated sulphuric acid was added gently and the mixture was shaken for 3 minutes. After that the acidic mixture was treated with a volume of ammonia for adjusting pH to 9 then the contents were put in separation funnel by adding 20 ml of chloroform for three times. The mixture was shaken well then two layers were formed and the aqueous layer was removed whereas organic layer having alkaloids, was gotten then it was dried and the weight of alkaloidic Extract was 1.45 grams [16]

### 2.3 Qualitative Detections Analysis of Alkaloids

The extracted alkaloidic compounds belonging to leaves of *Cordia myxa* were qualitatively tested by using various chemical reagents such as Dragendroft reagent for alkaloids, Molisch for carbohydrates, ferric chloride (1% w/v) for phenolic compounds, Benedict for glycosides, ethanolic potassium hydroxide (5N) for flavonoids, ninhydrin (1% w/v) for amino acids, mercuric chloride (5% w/v) for saponin and lead acetate (1% w/v) for tanninic compounds [17][18].

### 2.4 Culture Medium for pathogens

The pathogenic bacteria were treated with Muller-Hinton Agar (MHA) as a culture medium which was gotten from laboratories of marine science Centre in Basrah university-Iraq. It was prepared depending on all information's determined by company of manufacturing

#### 2.4.1 Pathogenic microorganisms in the study

The pathogenic microorganisms are bacteria strains are characterized to know the identity of all bacteria represented by *Staphylococcus aureus*, *Proteus sp.*, *Klebsiella sp.*, *pseudomonas aeruginosa* and *Escherichia coli*.

#### 2.4.2 Determination of Medicinal Efficacy of Alkaloidic Extract

The extracted alkaloids belonging to leaves of *Cordia myxa* were tested against pathogenic bacteria by using various concentrations then the maximum inhibitory concentration (150mg/ml) was carried out against these microorganisms by Agar-diffusion method by using many wells. Twenty mL of culture medium was put in petri dish then the medium was treated with 0.1 mL of bacterial suspension by using sterilized glass spreader. That 0.2 ml of Max IC belonging to alkaloids was added in each well. Finally the dishes were put in the incubator at 37 °C for one day then diameters of inhibition zones were measured [19].

## 3. Results and discussion

The increasing medicinal necessity for use of various potent chemical compounds extracted and identified from variety of herbs and plants led to increase the demands to get natural therapeutic drugs

from plants origin .So from these plants / *Cordia myxa* which is considered as one of feeding sources for human and animal together . Also abundance of many effective metabolic compounds ensured the importance of these secondary chemicals as natural therapies for different diseases caused by pathogenic microorganisms .Alkaloids were extracted successfully and purified with yield equal to 1.45 g from 25 g of *Cordia myxa* leaves therefore the extraction percentage was calculated as in table (1) .

**Table 1.** Extraction percentage of alkaloids extracted from leaves of *Cordia myxa*

| Plants leaves weight (g) | Alkaloidic Extract weight (g) | Extraction percentage (%) |
|--------------------------|-------------------------------|---------------------------|
| 25                       | 1.45                          | 5.8                       |

From above table , the extraction percentage was measured from the following law :

$$\text{Extraction percentage} = \frac{\text{Extract weight}}{\text{leaves weight}} \times 100$$

The percentage of extraction of alkaloids is somewhat very good because of existence of other secondary active compounds besides alkaloids specially phenolics, saponin, essential oils and flavonoids [20] . Therefore presence of alkaloidic compounds in *Cordia myxa* insures the bioactivity and medical efficacy of this plant . The variety of chemical reagents which were used to detect qualitatively on different secondary active metabolites was for insurance of existence of only alkaloidic compounds are present in the alkaloidic extract . Therefore orange precipitate was formed in good quantity by using Dragendroff reagent and appearance of this precipitate proves abundance of alkaloids in *Cordia myxa* leaves while the other active compounds showed a negative test as in table (2):

**Table 2.** Qualitative detections analysis of alkaloids extracted from *Cordia myxa* leaves

| Reagent type           | Result of detection | Chemical result         | Conclusion               |
|------------------------|---------------------|-------------------------|--------------------------|
| Dragendroff            | ++                  | Orange precipitate      | Alkaloids are present    |
| FeCl <sub>3</sub> (1%) | -                   | No green- bluish colour | Phenols are absent       |
| Molisch                | -                   | No violet ring          | Carbohydrates are absent |
| Benedict               | -                   | No red p.p.t            | Glycosides are absent    |
| Lead acetate (1%)      | -                   | No light brown p.p.t    | Tannins are absent       |
| HgCl <sub>2</sub> (5%) | -                   | No white p.p.t          | Saponins are absent      |
| Ethanolic KOH (5N)     | -                   | No yellow p.p.t         | Flavonoids are absent    |
| Ninhydrin(1%)          | -                   | No violet colour        | Amino acids are absent   |

The existence of alkaloidic compounds only in alkaloids fraction extract proves the accuracy of procedure which was carried out for extracted of alkaloids. The use of sulphuric acid in this method is for reaction with alkaloids because they have basic chemical features [18] . Because the *Cordia myxa* plant has a wide spectrum of potent chemical natural compounds therefore the medicinal efficacy of leaves belonging to this medical plant was investigated, determined and evaluated for Max IC with value equal to 150 mg/ml. So the all pathogenic bacteria used in the current research were underwent the Max IC of *Cordia myxa* leaves as indicated in table (3).

**Table 3.** Medicinal efficacy of alkaloids extracted from *Cordia myxa* leaves against some pathogens.

| Max. inhibitory Concentration (mg/ml) | Bacterial stains              | Inhibition zone diameter (mm) |
|---------------------------------------|-------------------------------|-------------------------------|
| 150                                   | <i>Staphylococcus aureus</i>  | 30                            |
| 150                                   | <i>Pseudomonas aeruginosa</i> | 26                            |
| 150                                   | <i>Proteus sp.</i>            | 25                            |
| 150                                   | <i>Escherichia coli</i>       | 22                            |
| 150                                   | <i>Klebsiella sp.</i>         | 30                            |

From table (3) , it was noticed that the maximum inhibitory concentration of alkaloids, has recorded the highest inhibition zone diameter against *Staphylococcus aureus* and *klebsiella sp.* Bacteria with value equal to 30 mm but the same Max IC has showed the lowest value of inhibition zone with diameter equal to 22 mm towards *Escherichia coli* bacteria . While the Max IC has given inhibition diameters with value equal to 25 and 26 mm towards growth of *Proteus sp.* and *Pseudomonas aeruginosa* respectively.

It was found that *Escherichia coli* showed the greatest resistance towards chemical action of alkaloids because this bacteria has dense lipidic layers in its cell wall then the alkaloidic extract was difficult to inter into the cell of this living microorganism. So the medicinal efficacy has decreased for killing this bacteria wheras the inhibition zone diameter was the highest against *Staphylococcus aureus* because this pathogen has less lipidic layers in this cell wall then the alkaloids can be inter easier into cell of this pathogenic bacteria therefore this extract could kill great number of this pathogen [21][22] .

The biochemical and medicinal potential of alkaloids can be explained by chemical bonding of these active metabolites with nucleic acids represented by DNA and RNA after that this process leads to chemical disorder in the metabolic pathways of these acids leading to inhibit the chemical roles and biological functions belonging to both acids [22][23] . Alkaloidic compounds can also destruct the enzymes which are responsible for biochemical anabolism of nucleic acids such as DNA polymerase and RNA polymerase [24] .

Various botanic studies represented the physiological and chemical properties of alkaloids as potent chemicals in inhibition the biochemical roles responsible for multi-metabolic pathways belonging to lipidic compounds, carbohydrates and proteins. The greatest functional group in alkaloidic compounds is imine which has chemical ability to destruct the pathogenic cell belonging to bacteria [25][26] .

The medicinal role of plants alkaloids can also belongs to capability of these natural metabolic compounds to do many chemical disorders in bio chemical anabolism process of proteins existing in the living cell of pathogenic bacteria leading to big deficiency in the different proteins of microorganism [27][28] .

#### 4. Conclusions

The extracted alkaloids belonging to Iraqi *Cordia myxa* leaves showed an excellent medicinal efficacy against growth of five pathogens represented by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus sp.*, *Escherichia coli* and *Klebsiella sp.* Bacteria. The maximum inhibition concentration (Max IC) proved the highest ability to inhibit or kill most pathogenic bacteria because the diameters values of inhibition zones were very good and they were insured by alkaloidic compounds which showed the high activity to finish or inhibit the biological and chemical systems of these pathogens. So the alkaloids extracted and purified from leaves of *Cordia myxa* can be carried out as natural active compounds for treating the various inflammatory caused by these pathogens instead of some synthetic drugs containing side effects.

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