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Evaluation of Inhibitory Activity of Cordia Myxa Fruit Extract on Microorganisms that Causes Spoilage of Food and Its Role in the Treatment of Certain Disease States

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Abstract
The study of chemical content and aqueous and alcoholic extracts of Aplomb's fruit and then detect the chemical components effective it aggregates and also, studying the effectiveness of inhibitory to extracts toward isolates bacterial different: E.coli, Pseudomonas flurscence, Salmonella, Shegilla and Staphylococcus aureus, where the results showed that the fruit Alpmbr containing 63.9% carbohydrate, 8.6% protein, 6.7% ash and 9.9% fat, so that the fruit Alpmbr high content of carbohydrates and fat shows that fat contains a high percentage of saturated fats, such as fatty acid, lauric acid ratio was 48.3% and Myristic acid was 15.8%, as well as that it contains a significant proportion of unsaturated fatty acid such as oleic by 12.4%, which is an important and basic nutrition and health for human. The fruit Alpmbr of mineral content was high proportion of potassium 29 ppm and sodium element was 13 ppm. It was found that either metallic zinc and calcium were accepted by Iraqi Standard. Heavy metals such as Cadmium, chromium and copper were not diagnose which is toxic elements, so it may be considered the fruit of Alpmbr is a safe fruits to-use and useful for health. To test the activity of the functional chemical components, it was found that high content of alkaloids, Saponin, Comarines, and Gums, and a reasonable rates of resins and Steroid, multiple phenols, flavonoids and Glycosides, tannins and oily nitrogeneous materials oily with low content. The pH of the aqueous extracts is 7.0 and alcoholic extract was 6.5 that means the fruits is neutral acidity. As for the inhibition activity toward the bacterial isolates were higher effective inhibitory concentration 200mg / ml of aqueous extracts a diameter of 26mm against Pseudomonas flurscence and 25 mm for the both of Salmonella and E.coli. While, it decrease significantly to 19mm towards both Shegilla and Staphylococcus aureus. But with alcoholic extract concentration 200mg / ml has decreased significantly to 25 mm diameter towards Staphylococcus aureus and 20mm in E.coli and Shegilla. Also the same significant decreases towards the Salmonella 19mm and 16mm towards the bacteria Pseudomonas flurscence. The sensitivity of the bacterial isolates for the antibiotics inhibition diameters was 20 mm, of Erthromycin against Salmonella. And the diameter of Oxacillin was 17 mm against Staphylococcus aureus, but Optimdex T was a very highly effective against all bacterial isolates.
Keywords: Cordia myxa-Chemicals content-Minerals-Bacterial inhibition activety

Introduction

Uses of medical plants are an important part of traditional medicine. One of these plants which is anciently used in Roman period from 2nd and 3rd centuries A.D. for its benefit properties is Cordia myxa (Karami, et al. 2015, Bouby, et al. 2011).

Cordia myxa fruit locally known as "Bumber" is one of the largest genera in the family Boraginaceae as about 300 species have been identified worldwide mostly in wormer region (Ali, et al. 2015, da Silva, et al. 2010). Common names include Lasura, Assyrian plim, Pidar, Panugeri, Naruvilli, Geduri, Spistan, Burgund dulu wanan and Ntege. It's found growing primary in Asia as well as across the globe especially in tropical regions having the right type of geophysical environment (Cordia myxa, 2016). *Cordia myxa. dichotoma* seeds are a good source of antioxidant agents available in everyday life (Shuge, T. et al. 2014). Cordia myxa is a sweeter fruit because it contains the maximum amount sucrose, glucose, fructose and high total dietary fiber, which plays one important role in decreasing risk of many diseases (Aberoumand, A. and Deokule S. 2010). Also, Cordia myxa fruit is a rich source of protein, fat , carbohydrates, ash, and essential minerals such as K, Na, Ca, Fe and Zn (da Silva, et al. 2010, Ali, 2011, and Karami, et al. 2015). Cordia myxa have high levels of glycosides, flavonoids, sterols, saponins , trepenoids, alkaloida, phynolic acids, gum and mucilage (Bhawana, P. et al. 2014, da Silva, et al. 2010 and Prasad G. 2013). So Cordia myxa fruit is popularly used for treatment of chest and urinary infections, Wound healing (Kuppast and Vasudeva 2006) and as an antihelminthic, diuretic, astringent, demulcent and expectorant agent, Moreover anti-inflammatory, and significant biological activities and anti-arthritic (Nawal, A. 2011, 1, 2, Walleda, et al. 2015, 4, 5, Abdallah, et al. 2011, 7 and). Therefore, this study focuses on detection of the components of Cordia myxa and evaluation inhibition activity against microorganisms.

Material and Methods

Material: The fruits were supplied from the Iraqi local market. After recognition, first it's carefully cleaned to

remove the dirt and extra genus materials, washed several times with running tap water, its seed removed out by pressing on seed, then dried in shade and finally powdered by grinding by using an electric blender. One hundred grams of dried powdered sample was extracted with 500ml of distilled water for 12 hours, and then filtered and phytochemical screening of the filtrate was carried out. Proximate analysis was carried out on dried powder of fruits.

Proximate composition: Proximate composition was carried out according to the procedure of Association of the Official Analytical Chemist (AOAC, 2005).

Fat content and fatty acids analysis:
Total fat was determined by the Soxhlet extraction method to obtained 200 mg. The extracted fat (80 ml) was transferred to esters, and concentrations of fatty acid s in the form of methyl-esters (FAMEs) were specified by gas chromatography analyzer GC-2010 (Shimadzu, Japan) with flame ionization detector (FID). The method with an addition of internal standard (C15:0) was used. The GC-2010 system was equipped with an auto sampler and auto injector. The injection volume was 1µl. The total split flow was 90 ml/min. Helium was used as the carrier gas. Air and hydrogen gases were used as auxiliary gases. The FAMEs were separated on a column VB-VAX (60 m length; 0.25 mm ID; 0.25µm film thickness). The FID temperature was set at 300^{o}C, initial injector temperature was 280^{o}C and pressure was 299.2 kPa, initial column temperature was 70^{o}C. The data were processed by a computer using data processor GC solution Post run (IUPAC, 1079).

Determination of calorie: The first total sugars content will be calculate, then total calorie value will be calculate, total calorie value is equal with fat calorie + protein calorie + sugars calorie. Each gram fat give 9 kcal, each gram protein give 4kcal and each gram sugar give 4kcal.

Mineral content: The sample was dry ashes at 550^{o}C and the ash boiled with 10ml of 20% HCl in a beaker and then filtered into a 100ml volumetric flask. It was made up to the mark with deionized solution and the mineral analysis of the resulting solution was determined using Atomic Absorption Spectroscopy (Pye, Unican SP9, Cambridge, UK).

Phytochemical screening:
Phytochemical screening procedures carried out were by the method of Odebiyi and Sowofora (1979). The crude C. myxa fruit extract (CME) was analyzed for PH, gum, alkaloids, saponin, steroids, polyphenols, flavonoids, comarines, resins, glycosides, tannin, and oily nitrogenous materials.

Extraction procedure
Aqueous extraction: Aqueous extract of *C. myxa* fruits were prepared according to (Sato *etal.,* 2000). *C. myxa* seeds were pulverized into fine powder using a stainless steel blender. Exactly 2 g of the powder (2 g) was placed in a 250 mL round-bottomed flask. Boiling water (100 mL) to conduct reflux extraction for 1h. The resulting solution was filtered while hot. The reflux extraction was repeated twice with 50 mL of boiling water for 0.5 h and filtered while hot. The filtrates from the three-reflux extractions were pooled and concentrated into 100 mL as the test solution.

Ethanolic extraction: *C. myxa* fruit were pulverized into fine powder using a stainless steel blender. The dried and powdered plant materials (3 g) were extracted once with 45 mL of 80% ethanol for 20 min by ultrasound. The ethanol extract was then filtered and ethanol was added to a final volume of 100mL.

Microorganism test:
Five bacterial strains, E. coli, Pseudomonas flurscence, Salmmonila, Shigella and Staphylococcus aureus were selected for the experiments.

Antibacterial activity E. coli, Pseudomonas flurscence, Salmmonila, Shigella and Staphylococcus aureus were grown in Mueller Hinton broth (Merck, Germany) at 37°C for 24h. Final cell concentrations were 10^{8} cfu/ml according to the McFarland turbidometry. 100µl of the inoculum was added to each plate containing Mueller Hinton agar (Merck, Germany). Three different concentrations of the C. myxa extract (50, 100, and 200mg/ml, respectively) were prepared. The sterile filter paper disks (6mm in diameter) were saturated with 50µl of each concentration of the extract. The plates were incubated at 37°C for 24h and the diameters of inhibitory zones were measured. The assay was carried out three times for each extract. Disks containing different concentrations of antibiotics were used as reference to compare the sensitivity of each tested bacterial species (Hsieh, et al. 2001). Antibiotics disks contain Erythromycin, oxacillin and Optimdex T with 15mg, 1mg and 1mg concentration respectively (Bact Difco, USA).

Statistical Analysis
The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Result and Discussion

Table 1 showed the result for the proximate analysis of *Cordia myxa* fruits. It shows that it contains 63.9% of carbohydrate, 8.6% protein, 6.7% Ash and 9.9% fat. Carbohydrate has the highest value while ash content had the least value. The results obtained from the proximate analysis of *Cordia myxa* fruits established the fact that it can be ranked as carbohydrate, fat, protein and ash rich fruit due to their relatively high content. These results can favourably compare with that reported by Iniaghe et al., 48.48% Carbohydrates and 2.5 % crude fat (Aberoumand, 2011).

Table 1: Chemical Composition of *Cordia myxa* Fruit.

Contents Value	Percentage
Moisture	**10.9±0.52**
Ash	**6.7±018**
Protein	**8.6 ±0.62**
Fat	**9.9±0.16**
CHO	**63.9±0.41**
Calorie	**3.79±0.07**

Values expressed as mean±SD of three determinations

Fat and fatty acid contents:

Fat and fatty acid content presented in table (2). This study showed that high content of fat 9.9%, higher than measured by (Ali, and Deokulem 2009, Ali, 2011). The high percent of fatty acid in Iraqi C.myxa is the saturated fatty acid C_{12} and C_{14} which its percent were 48.3 and 15.8 respectively. In addition of that C.myxa contain a reasonable percent of unsaturated fatty acid, oleic acid 12.4 that's very important for health and nutrition for consumers.

Table 2: Concentrations of fatty acids most frequently present in oil of *Cordia myxa* fruits powder

Fatty acids	g/100g^{-1}oil)%(
C_6	**0.1684**
C_8	**3.1862**
C_{10}	**3.3679**
C_{12}	**48.3092**
C_{14}	**15.7670**
C_{16}	**10.7059**
C_{18}	**2.7517**
$C_{18:1}$	**12.3774**
$C_{18:2}$	**1.6611**
Fat (g/100g^{-1})	**9.9**

Note: Data expressed as T-test * ($p < 0.05$), NS: Non-significant. namely C_6 Caproic acid, $C:_8$ Caprlic acid, C_{10} Capric acid, C_{12} lauric, C_{14} Myristic, C_{16} palmitic acid, C_{18} Stearic, $C_{18:1}$ Oleic, and $C_{18:2}$ Linoleic.

Mineral analysis result: (Table 3) presented that potassium 29 ppm, and sodium 13 ppm is the most abundant minerals present in the fruits. Other minerals detected in reasonable amounts are Iron and Magnesium, which were approximately 0.5 and 0.2 ppm respectively. While, Calcium, Manganese and copper were 0.07, 0.02 and 0.01 ppm respectively. Calcium helps in bone formation and blood coagulation. Sodium is the main inorganic cation of extracellular tissue fluids, potassium function principally as the cation of the cell and also in nerve and muscle excitability. Iron is important as a constituent of haem, an essential part of haemochromagens important in respiration. . Lead, cadmium, chromium and copper were not detected in the fruits and this is of great advantage to the consumers since these heavy metals have been reported to be toxic (Okwu, 2001). This finding is accordance to finding by (Aberoumand, 2011).

Table 3: Mineral Composition of *Cordia myxa* Fruits

Minerals concentration	Ppm
Na	**13**
K	**29**
Ca	**0.0734**
Fe	**0.4659**
Mg	**0.1431**
Mn	**0.0298**
Cu	**0.0116**
Zn	**0.0072**
Pb	**0.0015**
Co	**-0.0274**
Cd	**-0.0011**

N.D.: Not Detected -

Phytochemical screening

The results of the Phytochemical screening were presented in Table (4), showed the presence of Alkaloids, Saponin, poly phenols and Comarine were high contents. While, Steroid, resine, glycosides, tannins, and gum were low contents. Presences of these phytochemicals have contributed to its medicinal value as well as physiological activity (Lewis, et al. 1977). Polyphenols component have been shown to have antibacterial, anti-inflammatory, ant allergic, antiviral and antineoplastic activity (Alan, 1969). Many of these alleged effects, due to their known functions as strong antioxidant and metal chelates (Elegbede, 1998). Steroidal compounds are of importance in pharmacy because of their relationship with such compounds as sex hormones (Bruneton, 1999). Saponins have been reported to be linked with tumor inhibiting activity on animals. The positive effects of glycoside and cardiac glycoside are not common but their toxic effects were decreased heart rate, symphatetic activity and systematic vascular resistance (Sofowra, 1993).

Table4: Phytochemical Screening of *Cordia myxa Fruits.*

Chem. Tests	Aqueous extract	Alcoholic extract
PH	**7.0**	**6.5**
Gum	+	+
Alkaloid	+++	++
Saponine	+++	+
Steroid	+	+
Polyphenols	+	+
Flavonoids	+	++
Comarines	++	+
Resins	+	+++
Glycosides	+	+
Tannins	+	+
Oily nitrogeneous material	+	+

Where '++, +++' means highly available and '+` means low available

Antibacterial activity

Antibacterial activity of aqueous extract of C. myxa is showed in table (5). It was found that bacterial inhibition zone enhanced significantly with increased of the fruit extract concentration as shown clearly in the following table. The maximum antibacterial activity in the aqueous extract 200 mg/ml concentration of the C. myxa was 26mm for Pseudomonas flurscence and 25mm for Salmmonila, 24mm for E.coli with resistance. Also the bacterial activity decreased to 19mm with resistance for both Shigella and St. a. While the antibacterial activity of alcoholic extract of C. myxa is lowered than aqueous extract of C. myxa as shown in table (5) for all strains of bacteria that's used in this study.The maximum antibacterial activity in 200 mg/ml concentration of alcoholic extract C. myxa was 25mm for Staphylococcus aureus with resistant, 20mm for both E.coli and Shigella. Then the bacterial activity decreased significantly to 19mm with resistance for Salmonella and 16mm for Pseudomonas flurscence. This finding comparable to (Somayeh, et al. 2011).

Table (5): Inhibition zone (mm) Cordia myxia extracts (aqueous and alcoholic) at different concentration (Mean±SD)

Concn. / Bacteria	50mg/ml		100mg/ml		200mg/ml		CD.W.	LSD value
	Aq Mm	Alc mm	Aq mm	Alc mm	Aq mm	Alc mm		
E. coli	10 R	6	13 R	11 R	24 R	20 R	0	5.42 *
Pseudomonas flurscence	10	8	14	7	26	16	0	4.96 *
Salmmonila	11	7	18 R	10	25 R	19 R	0	4.63 *
Shigella	9 R	14	11 R	17	19 R	20	0	4.06 *
Staphylococcus aureus	10 R	8 R	20 R	18 R	19 R	25 R	0	5.31 *

R= Resistant, Aq=Aqueouse extract, Alc= Alcoholic extract, C=Control of distilled water.

Antibiotic resistant:

Field existences of antibiotic resistant pathogenic bacteria are increasing in recent years. Pharmaceutical companies are now looking for alternatives. Plants have been a rich source of medicines because it is believed that plant based drugs cause less or no side effect and affect a wide range of antibiotic resistant microorganisms. This study showed that E.coli, Pseudomonas flurscence, Salmmonella, Shegilla and Staphylococcus aureus bacteria were used against to the Erythromycin15 mg, Oxacillin 1mg and Optimdex T antibiotics as shown in table 6. It was found that E.Coli bacteria had significantly high inhibition zone 27mm to the Optimdex T antibiotic, then 11mm zone with Erthromycin 15mg. But there were no effect of inhibition to the Optimdex 1mg that was zero mm. The same thing with Pseudomonas flurscence bacteria, there were significant differences of inhibition zone to the same different antibiotics. Thirty four mm of inhibition zone to the Optimdex T antibiotics, then decreased significantly to 12mm with Erthromycin 15mg and zero mm inhibition with oxacillin antibiotics. Also with Salmmonila a high significant differences of inhibition with same different antibiotics. 40mm, 20mm and 8 with resistance to Erthromycin 15mg, oxacillin 1mg and Optimdex T respectively. The same thing happen with Shegilla and Staphylococcus aureus bacteria as shown clearly in table 6.

Table 6: Inhibition zone (mm) of antibiotic disks (mean ± SD)

Antibiotic disks	Inhibition diameter (mm)				
	E.coli	PF	S	Sh	St
Erythromycin 15 mg	11	12	20	10	2
Oxacillin 1mg	Zero	Zero	8 R	Zero	17 R
Optimdex T	27	34	40	27	26
LSD value	5.75 *	5.46 *	4.21 *	5.07 *	4.88 *

E. coli, Pf= Pseudomonas flurscence, S=Salmmonila, Sh=Shigella and St=Staphylococcus aureus, R=Resistant

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تقييم الفعالية التثبيطية لمستخلص فاكهة البمبر في الاحياء المجهرية المسببة لتلف الاغذية ودورها في علاج بعض الحالات المرضية

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الملخص
تم دراسة المحتوى الكيمائي والمستخلصات المائية والكحولية لثمرة نبات البمبر ومن ثم الكشف عن المكونات الكيمائية للمجاميع الفعالة لها وبعد دراسة الفعالية التثبيطية لهذة المستخلصات تجاه عزلات بكتيرية مختلفة: E.coli, Pseudomonas flurscence, Salmonella, Shegilla and Staphylococcus aureus , حيث اظهرت النتائج ان ثمرة البمبر تحتوي 63.9% كربوهيدرات, 8.6% بروتين, 6.7% رماد و 9.9% دهون, وذلك يبين بان ثمرة البمبر عالية المحتوى من الكربوهيدرات والدهون وان الدهنيات تحتوي على النسبة العالية من الدهون المشبعة مثل حامض اللوريك نسبة 48.3% وحامض المايرستك بنسبة 15.8%, فضلا عن ذلك إحتوائها على نسبة لا بأس بها من الحامض الدهني الغير مشبع الاوليك بنسبة 12.4% وهو من الدهون المهمة والاساسية للتغذية وصحة الانسان. أما محتوى ثمرة البمبر من المعادن فقد كانت عالية النسبة بالبوتاسيوم ppm2913 وعنصر الصوديوم بنسبة ppm ولم , اما معدني الزنك والكالسيوم فكانت بنسبة مقبولة, يشخص فيها كلا من العناصر الثقيلة كالكادميوم, والكروم والنحاس حيث أنها من العناصر السامة , لذا يمكن إعتبار ثمرة البمبر من الفواكهة الآمنة للاستعمال والمفيدة للصحة. أما بالنسبة للمكونات الكيمائية للمجاميع الفعالة فقد كانت عالية المحتوى من القلويدات والصابونينات والكومارينات وبنسب معقولة من الراتنجات والصمغ وبنسب قليلة من الستيرودات والفينولات المتعددة والفلافونيدات والكلايكوسيدات والتانينات والمواد النيتروجينية الزيتية, وأن الاس الهدروجيني للمستخلص المائي هو 7.0 وللمستخلص الكحولي فكان 6.5 أي انها من الثمار المتعادلة للمستخلص المائي وهي 200mg/ml الحموضة. أما فيما يخص الفعالية التثبيطية تجاه العزلات البكتيرية فقد كانت أعلى فعالية تثبيطية بتركيز

19mm وتنخفض إحصائيا الى E.coli و Salmonellaلبكتريا 25mm وPseudomonas flurscence لكل من بكتريا26mmبقطر فقد إنخفض إحصائياً قطر 200mg/ml. أما بالنسبة للمستخلص الكحولي وبتركيز Staphylococcus aureus و Shegilla تجاه كل من ويستمر الانخفاض Shegilla و E.coli تجاه كلا من بكتريا 20mm و Staphylococcus aureus تجاه بكتريا 25mmالتثبيط الى . أما حساسية العزلات البكتيرية للمضادات Pseudomonas flurscence تجاه بكتريا 16mm و Salmonella تجاه 19mmالإحصائي الى تجاه 17mm فقد كان قطر تثبيطه هو Oxacillin وأما 20mm هو Salmonella تجاه بكتريا Erthromycinالحياتية فقد كان قطر تثبيط فقد كان ذو فعالية عالية جداً تجاه عزلات البكتريا كلها.Optimdex T ولكنStaphylococcus aureusبكتريا

الكلمات المفتاحية: فاكهة البمبر-المحتوى الكيمائي- المعادن-الفعالية التثبيطية للبكتريا

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