

Comparative study of *In vitro* inhibitory effect ethyl and methyl extracts of Dom Palm *Hyphaene thebaica* fruit on bacteria .

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Summary:

The comparable of antimicrobial activity of ethyl and methyl extracts of Doum palm *Hyphaene thebaica* fruit were studied on gram positive and gram negative bacteria . The minimum inhibitory concentrations (MICs) of extracts were determined . The more effective extract on all microorganisms was ethyl extract when compared with methyl extract . the more sensitive microorganisms to ethyl and methyl extract were *Saphylococcus aureus* which have inhibition zone (28,21)mm respectively ,while *Pseudomonas aeruginosa* have less inhibition zone(10,8)mm for extracts from other bacteria .

Introduction

Herbal medicine has a long history in the treatment of several kinds of diseases (Fletcher, 1997). This development has also led to focusing research activities toward the chemical and microbiological loads of herbal medicinal products (Von, 1986). The Doum palm ; *Hyphaene thebaica* mart fruits is growing wild throughout the dry regions of tropical Africa , the Middle East and Western India. The fruits of Doum showed antimicrobial and antihypertensive activities ,these activities were attributed to the presence of flavonoids , phenols& glycosides compounds (Pieroni *et al*,1996 and Burkill,1997).

Also the aqueous extract of Doum fruits showed an antimicrobial activity , this is due to water soluble phenolic contents .

The aim of present study was to evaluate antimicrobial activity of *Hyphaene thebaica* fruit extract against some bacterial pathogens .

Materials and methods

The mesocarp was removed with a knife and air dried at air temperature until it became hard after at least 10 days. The resultant dried fruit was ground to yield powder material which was then used for extraction (Alarcon-Aguilar *et al*, 1997).

Bacterial isolates

Clinical isolates of human pathogenic bacteria were obtained from the microbiological laboratory unit of Basrah general Hospital which include the gram negative bacteria (*Escherichia coli*, *proteus vulgaris* and *Pseudomonas aeruginosa*)and gram positive bacteria (*Staphylococcus aureus* and *Streptococcus faecalis*) .

Medium

Nutrient broth , nutrient agar and Mullere Hinton agar were used in this assay. Methanol acetate and ethyl acetate were used as solvents to dissolve the extract . the serial dilutions of the resultant extract solution were made to reach the concentrations of (25,50,75,100)Mg ml⁻¹ for determinations of Minimum Inhibitory Concentrations (MICs).

Methyl extraction

Thirty gram of the dried powder of the fruit *Hyphaene thebaica* was taken and put in a thimble. The method of Soxhlet continuous extraction was performed for 16 hours using 250 ml of methanol as a solvent. After evaporating the solvent a brown color viscous material of 10 grams weight was obtained (Alarcon-Aquilar *et al*, 1997).

Ethyl extraction

A 30 grams of dried powder of the fruit *Hyphaene thebaica* was put in thimble. Continuous extraction using the method of Soxhlet extraction was performed using 250 ml of ethyl acetate for 16 hours. The resultant was yellowish-to-brown viscous material weighing 10 grams (Alarcon-Aquilar *et al*, 1997).

Measurments of Minimal Inhibitory concentrations of methyl and ethyl extracts

Sterile Muller Hinton Agar (MHA) plates were prepared. To each plate was added a volume of 2ml of nutrient broth containing standard strains of either gram positive bacteria (*Staphylococcus aureus* and *Streptococcus faecalis*), or gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *proteus vulgaris*). L-shaped glass rod was used to streak the bacteria in the nutrient broth solution on the surface of the agar plate. Small discs impregnated with the two extract types namely the methanol extracts and the ethyl extracts were placed on the agar in the following concentrations for each (25,50,75,100,and 125 mg/ml). The plates were then incubated for 24 hours at a temperature of 37°C. After that time, the plates were examined for areas in the agar in which there is void of bacterial growth around the extract soaked discs (i.e. inhibition zone). The diameter of each inhibition zone was measured and the measurements were expressed in millimeters (mm) (Collee *et al*, 1996).

Results:

Table (1) shows the results of the minimal inhibitory concentrations (MICs) for ethyl and methyl extracts of *hyphaene thebaica* fruit on gram positive and gram negative bacteria. Using for concentrations for each extracts (25, 50, 75and 100 mg/ml).

The least extract concentration (ethyl and methyl) to which bacterial sensitivity occurred was 50 mg/ml. The inhibition zone increased as the concentration of the extract had increased to 75 mg/ml and still further

increase in the inhibition zone when the extract concentration reached 100 mg/ml .

No single bacteria was found to be inhibited at extract concentration of 25 mg/ml.

The pattern of bacterial sensitivities to the type of extract was as follows :

Ethyl extract :

At extract concentration of 100 mg/ml , *Staphylococcus aureus* was found to be the most sensitive (inhibition zone :28mm), followed by *Streptococcus faecalis* (inhibition zone : 20mm), the least sensitive ethyl extracts were gram negative bacteria with inhibition zones (10,13, 16) mm for *Pseudomonas aeruginosa* , *Proteus vulgaris* , *Escherichia coli* respectively .

Methyl extract :

At extract concentration of 100 mg/ml the inhibition zone to gram positive bacteria is still greater than to gram negative bacteria.

Staphylococcus aureus was found to be the most sensitive with inhibition zone of 21 mm followed by *Streptococcus faecalis* with 18 mm inhibition zone .

For gram negative bacteria the inhibition zones were (8,9 and 11) mm for *Pseudomonas aeruginosa* , *Proteus vulgaris* and *Escherichia coli* respectively .

Table (1):Determination of minimal inhibitory concentration of ethyl and methyl extracts on bacteria

	Minimal inhibitory concentration mg/ml							
	Ethyl extract				methyl extract			
MICs mg/ml	25	50	75	100	25	50	75	100
Microorganisms	Inhibition zone of bacteria mm							
<i>Staphylococcus aureus</i>	0	20	25	28	0	14	19	21
<i>Streptococcus faecalis</i>	0	10	17	20	0	9	12	18
<i>Escherichia coli</i>	0	9	11	16	0	5	9	11
<i>Proteus vulgaris</i>	0	7	9	13	0	5	7	9
<i>Pseudomonas aeruginosa</i>	0	5	8	10	0	3	6	8

Discussion :

This study showed that both ethyl and methyl extracts of the fruit *Hyphaene thebica* had reasonable antibacterial activity against both gram positive and gram negative bacterial strains tested in the study . The ethyl extract was found to be have greater antimicrobial activity against both gram positive and gram negative bacteria as compared to methyl extract.

The main reasons for this difference in inhibitory effect of ethyl and methyl extracts of *Hyphaene thebicae* fruit on gram positive and gram negative bacteria is attributed to the difference in cell wall of gram negative bacteria have high percentage of lipids. These compounds play a role in preventing the reach of adequate concentration of the inhibitory compounds of the extracts to the inside of bacterial cells and thus rendering these gram negative bacteria less susceptible to these compounds (Collee *et al*, 1997; Heimler *et al*, 1992 and Grosenror, 1995).

The study had also demonstrated that generally the degree of bacterial inhibition was greater with ethyl extracts as compared to methyl extracts.

This variation in antimicrobial effect is attributed to the difference in the amount of effective compounds that can be extracted or purified from the tested plant ((Alarcon-Aquilar *et al*, 1997) .

The ethyl extraction of *Hyphaene thebicae* yields flavoniods and other activity compounds which have a very important role in inhibition of microorganisms. This inhibition is due to precipitation of microbial protein due to formation of hydrogen bonds between groups of aromatic hydroxide and microbial protein leading to inhibition of important enzymes necessary for metabolism activities of the microorganisms (Gauon, 1972).

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دراسة مقارنة التأثير التثبيطي لخلاصة الأثيل والمثيل لثمرة نخل الدوم *Hyphaene thebaica* على البكتريا .

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الخلاصة

تم دراسة التأثير التثبيطي لخلاصة الأثيل و المثيل لثمرة نخل الدوم *Hyphaene thebaica* على البكتريا الموجبة والسالبة لصبغة كرام. حدد التركيز المثبط الأدنى للمستخلصين على البكتريا، وكان تأثير خلاصة الأثيل أكثر فعالية على الجراثيم عند مقارنتها مع خلاصة المثيل حيث أظهرت جرثومة *Saphylococcus aurues* أكثر الجراثيم تحسناً لكلا المستخلصين وبمنطقة تثبيط (٢١،٢٨) ملم على التوالي . في حين أظهرت جرثومة *Pseudomonas aeruginosa* أقل الجراثيم تحسناً وبمنطقة تثبيط (٨،١٠) ملم لكلا المتخلصين على التوالي.