

## EVALUATION OF ANTIMICROBIAL ACTIVITY OF PHENOLIC EXTRACT FROM *Haloxylon* *salicornicum*

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### Abstract

Investigation of antibacterial and antifungal activity of phenolic extract of *Haloxylon salicornicum* was carried out in this study on Gram positive and Gram negative pathogenic bacteria and some species of fungi. The results exhibited variable susceptibilities of microorganisms for different concentration of phenolic extract. The activity of this extract was associated with high concentrations. Using plate method, phenolic extract of *H. salicornicum* had the highest effect and wide diameter of growth inhibition zone against *Candida albicans* and *Klebsiella pneumonia*, and it has no effect on growth of *Aspergillus fumigatus* and *A. terreus* only when very high concentrations is used.

### Introduction

A new wave of research interested in traditional practices which might be used as antimicrobial has been stimulated by the renewed attention to natural therapies. Microbiologist have two reasons to be interested in topic of antimicrobial plant extracts. First, it is very likely that these phytochemical will find their way in to arsenal of antimicrobial drugs prescribed by physicians. New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problem with the over prescription and misuse of traditional antibiotics<sup>(1)</sup>.

*H. salicornicum*, a plant belongs to the family Chenopodiaceae, grow as a small herb and cultivated in desert in Iraq, Syria, Iran, Egypt, Pakistan and Afghanistan. *Haloxylon* is used as folk medicine such as *antiulcer*<sup>(2)</sup>, diuretic activity<sup>(3)</sup> hypoglycemic and antibacterial<sup>(4)</sup>. The plant contains phenols compound, which was isolated and identified as an antibacterial substance by Brantner and Grein<sup>(5)</sup>. Phenyl propane-derives compounds which are in highest oxidation state. Two types of alkaloids compounds of *H. salicornicum* have been isolated, betain and piperidine.

However, antimicrobial evaluation of phenols compound from *H. salicornicum* not understood, therefore, we examined the antimicrobial activity of *H. salicornicum* extract on bacterial and fungal growth.

### Material and Methods

#### Plant material and extraction:

The plant of *H. salicornicum* were purchase from the local market of Basrah. The plant was grind or crushed to power (20g) which was used for phenols extract with acetic acid (400ml) using reflex condenser below 70°C for 8 hrs and let solution to cooled. Solution was separated by centrifugation and put below part in separation funnel and equilibrium of n-propane and NaCl for were added to saturation, this revealed two layer which contain phenolic compounds which was concentrated under reduced pressure by a rotary evaporation below 40° (6)

### Microorganisms Test:

Six types of pathogenic bacteria & five species of fungi were previously isolated and identified by other works. To study the antimicrobial activity of phenolic extract of *H. salicornicum*, Muller-Hinton agar medium was used for bacterial growth, plates were incubated at 37°C for 24-48 hrs. For fungal growth ; potato dextrose agar (PDA) medium was used for growth of *Candida albicans* and sabroud dextrose agar (SDA) was used for growth of *Aspergillus* species. Plates were incubated at 25°C for 3-5 days.

In both cases the method of well contain extract were used and the inhibition zones were measured by scale and compared with the control<sup>(7)</sup>.

### Results:

The results of the study are summarized in Table 1. The table shows the means of diameter of inhibition zone induced by phenolic extract of *H. salicornicum* on the growth of microorganisms. The inhibition zones induced by extract also illustrated by photographs which are listed in figure 1 (A-F) and figure 2 (A-E). Table 1 reveals different influence of extraction on microorganisms due to different concentration of this extract. The phenolic extract is strongly inhibit the growth of many types of bacteria above the concentration 500 mg/ml . The growth of *K. pneumoniae* is inhibited with less concentration (200 mg/ml). In contrast, the growth of *B. subtilis* and *E.coli* is less inhibited even with high concentration. Fungi showed different ability of resistant against extract, *C. albicans* is the more effected microbe by the extract even with lower concentration. *A. niger* and *A. flavus* showed low inhibition by the extract, but the extract has no effect on growth of *A. fumigatus* and *A. terreus* only when concentration increased to 2000 mg/ml (the result not show in the table).

### Discussion

Present study exhibited importance medicinal of the *Haloxylon* plant through antimicrobial activity of the phenolic extraction. Microbs showed a variable susceptibility for different concentrations of phenolic extract. Some of simplest bioactive phytochemical consist of single substituted phenolic ring which are in the highest oxidation state. The common herbs contain phenols, which is effective against bacteria<sup>(8&9)</sup> and fungi<sup>(10)</sup>. Hydroxylated phenols shown to be toxic to microorganisms. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity<sup>(11&12)</sup>. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compound, possibly reaction with sulfhydryl groups or through more nonspecific interaction with the proteins<sup>(13)</sup>.

In this study we observed that the studied gram positive pathogenic bacteria were high susceptible more than gram negative pathogenic bacteria. In spite of presence some exceptional, it may be due to cell membrane of gram positive pathogenic bacteria which composed from peptidoglycan, mucopolysaccharids and phospholipids. This will provide suitable medium for possibility to interaction and acts as bactericidal or bacteriostatic agents and give rise to affect as destructive whether on membrane or on building unit of protein structure or nucleic acid synthesis inside the bacterial cell. Comparative with gram negative bacteria the cell membrane of these bacteria composed from two membranes, outer and inner membrane and separated by the periplasmic space. The outer membrane composed of three materials, mucopolysaccharides, Lipoproteins and phospholipids, while inner membrane composed from peptidoglycan and glycopeptides. The cell membrane of gram negative bacteria contain 90-95% lipids. These contains were not provided

suitable medium to reaction with extracts. Therefore gram negative bacteria is less susceptible to phenolic extract due to the morphology of cell membrane. In addition to that, antifungal activity of phytochemicals can be assign to spore degeneration by phenolic extract or it might effect on spores germination. . These result agree with<sup>(4)</sup>.

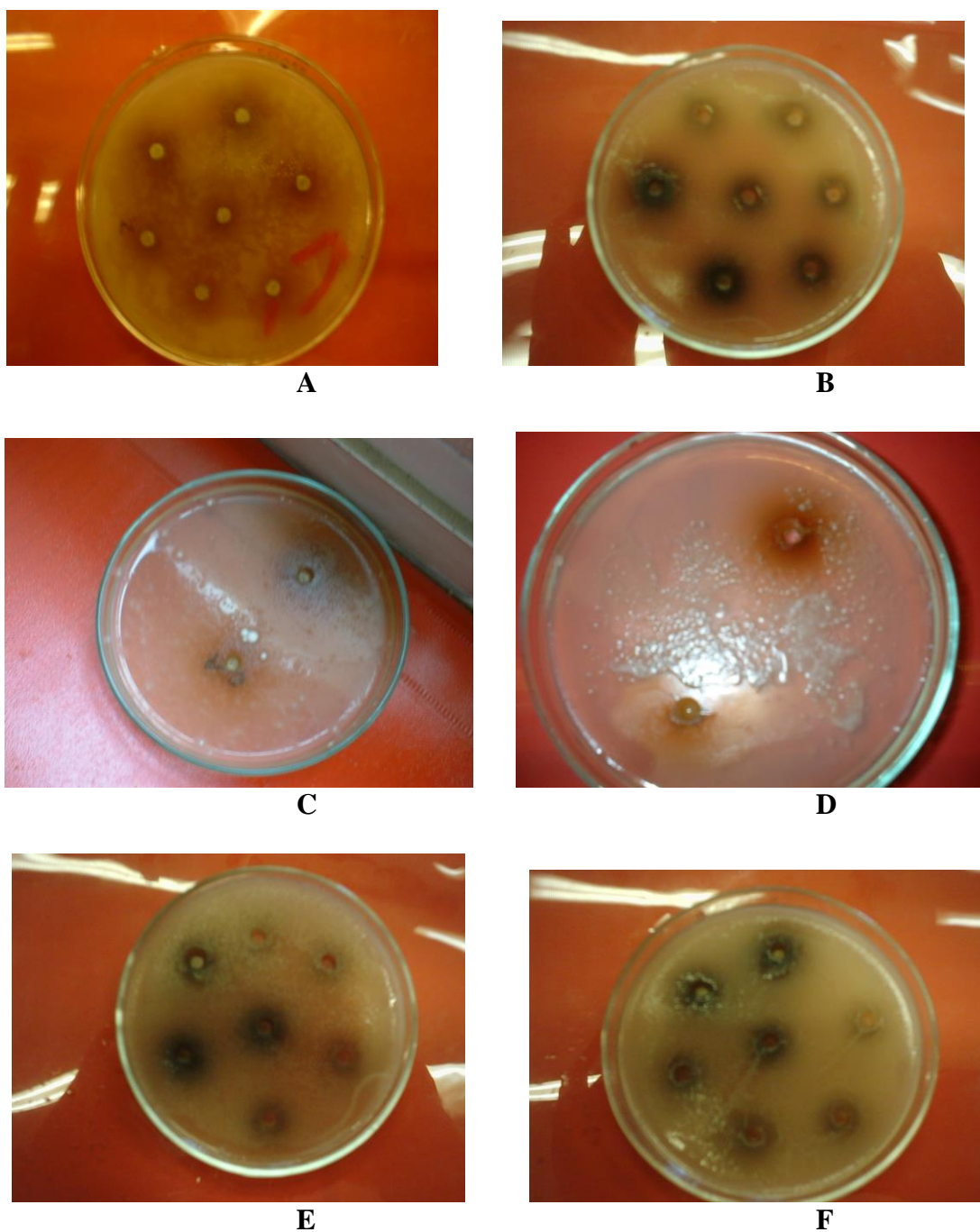
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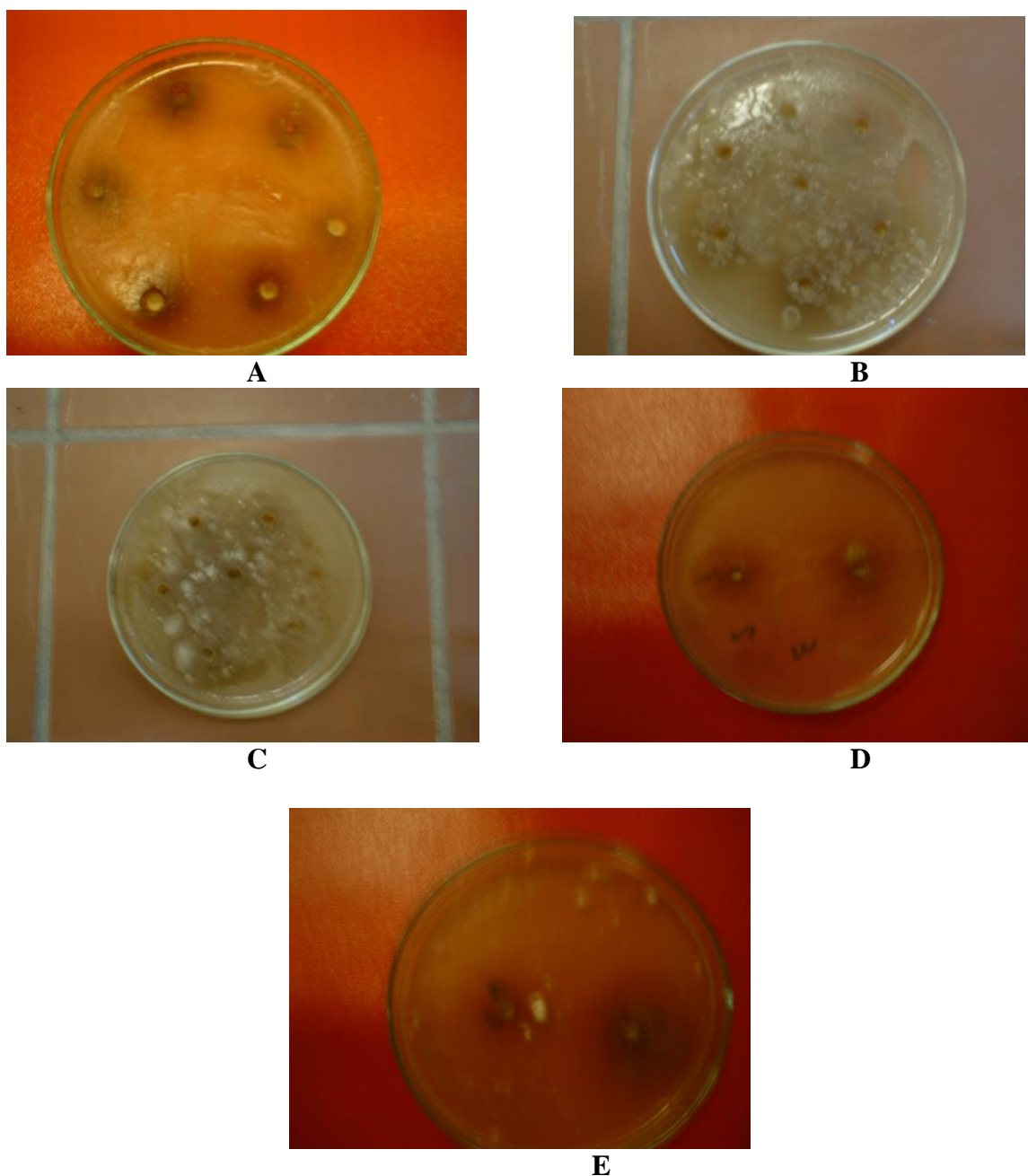
**Table.1 Mean of diameter of the inhibition zones induced by phenolic extract on microorganisms used in this study**

Microorganism	Concentration of phenolic extraction (mg/ml)						
	200	300	400	500	600	800	1000
	DIZ (mm)	DIZ (mm)	DIZ (mm)	DIZ (mm)	DIZ (mm)	DIZ (mm)	DIZ (mm)
<b>Gram positive</b>							
<i>Bacillus subtilis</i>	2	2	5	8	9	9	-
<i>Staphylococcus epidermidis</i>	2	3	9	9	10	13	15
<i>Streptococcus sp.</i>	3	3	2	13	14	14	15
<b>Gram negative</b>							
<i>Escherichia coli</i>	3	4	4	5	9	9	-
<i>Klebsiella pneumoniae</i>	10	10	10	10	15	15	15
<i>Pseudomonas aeruginosa</i>	2	3	5	8	10	13	13
<b>Fungi</b>							
<i>Candida albicans</i>	10	13	15	17	18	18	20
<i>Aspergillus niger</i>	2	2	3	3	4	5	6
<i>Aspergillus flavus</i>	2	3	4	4	5	6	7
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	-
<i>Aspergillus terreus</i>	-	-	-	-	-	-	-

DIZ= Diameter of Inhibition Zone measured in millimeter.



**Figure 1. Inhibition zones induced by phenolic extract on growth of some bacterial species. A. *Bacillus subtilis*, B. *Staphylococcus epidermidis*, C. *Streptococcus sp.*, D. *Escherichia coli*, E. *Klebsiella pneumoniae*, F. *Pseudomonas aeruginosa***



**Figure 2. Inhibition zones induced by phenolic extract on growth of some fungal species. A. *Candida albicans*, B. *Aspergillus niger*, C. *Aspergillus flavus*, D. *Aspergillus fumigatus*, E. *Aspergillus terreus*.**

#### الخلاصة

لقد تم دراسة الفعالية المضادة للميكروبات للمستخلص الفينولي لنبات الشنان. وقد اختيرت بعض انواع البكتريا المرضيه الموجهه والسالبه لصبغة جرام. كما تم دراسة تأثير المستخلص على بعض انواع الفطريات ايضا. بينت الدراسه ان هذه الميكروبات لها حساسيه مختلفه تجاه المستخلص وحسب نوع الميكروب والتركيز المستخدم. وقد لوحظ ان التأثير الاكبر للمستخلص كان على جرثومة *Klebsiella pneumoniae* وفطر *Candida albicans*. اما الفطرين *Asprigillus fumigatus* و *Aspergillu. terreus* فلم تتأثر بالمستخلص الا عند استخدام التراكيز العاليه جدا.