

## The Antibacterial Activity of *Callemia Sinensis* (green tea) on Some Bacterial Species.

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### الخلاصة:

الهدف الاساسي من البحث هو تقييم التأثير التثبيطي للمستخلص الكحولي للشاي الأخضر لإيجاد التأثير التثبيطي ضد البكتريا الموجبة و السالبة لصبغة كرام. تم إستخلاص الزيت الفعال من أوراق الشاي الأخضر بأستخدام الميثانول و أختبرت فعاليته ضد 54 عزلة بكتيرية معزولة من إصابات الجلد، إصابات المجاري البولية و إصابات الجهاز التنفسي من خلال طريقة الأنتشار في الأكار. أظهرت النتائج أن للمستخلص الكحولي للشاي الأخضر تأثير تثبيطي على 54 عزلة بكتيرية تم عزلها في هذه الدراسة: 8 عزلات لبكتريا *Escherichia coli*، و 8 عزلات لبكتريا *Streptococcus pneumoniae*، و 12 عزلة من بكتريا *Klebsiella pneumoniae*، و 8 عزلات لبكتريا *Staphylococcus aureus*، و 6 عزلات لبكتريا *Proteus species*، و 12 عزلة من بكتريا *Pseudomonas aeruginosa*. إن الشاي الأخضر يمتلك فعالية تثبيطية كافية و يمكن أن يستخدم كعلاج كيميائي من خلال معرفة ميكانيكية عمله لغرض استخدامه كعلاج وقائي.

### Abstract:

The main objective of this research is to screen and to evaluate the antibacterial activity of methanol extract of *Callemia Sinensis* and to find out zone of inhibition against gram positive (G+ve) bacteria as well as gram negative (G-ve) bacteria.

Extraction of the leaves *Callemia Sinensis* (green tea) and tested against 54 isolates of G+ve and G-ve bacteria which were isolated from different samples, which include pus of skin, urine and throat swabs of patients who were admitted to Al-jerहत specialized hospital in Baghdad during the period of 1/12/2008 to 1/7/2009.

The methanolic extract of the plant *Callemia Sinensis* (green tea) showed antibacterial activity against 54 bacterial isolates that would be isolated in this study: 8 isolates of *Escherichia coli*, 8 isolates of *Streptococcus pneumoniae*, 12 isolates of *Klebsiella pneumoniae*, 8 isolates of *Staphylococcus aureus*, 6 isolates of *Proteus species*, and 12 isolates of *Pseudomonas aeruginosa*.

*Callemia Sinensis* have been shown to have potential chemotherapeutic activities. These data provide a direct mechanism of action for *Callemia Sinensis* and further support its role as a chemopreventive agents.

**Key words:** *Callemia Sinensis*, antibacterial activity, G+ve bacteria, G-ve bacteria.

### **Intro daction:**

*Callemia Sinensis* (green tea) is rich in the polyphenolic compounds-bounded benzene rings with multiple hydroxyl groups, and non flavonoid. Tea polyphenols are strong antioxidant, improve gastrointestinal function and alcohol metabolism, it improve, kidney, liver and pancreatic function, protect skin and eyes, and alleviate arthritis<sup>[1]</sup>.

Tea has been used in managing and preventing allergies, diabetes, viral infection, tooth cavities to reduce acute disease with an inflammatory component and to improve psychological health<sup>[2,3]</sup>.

Hexane a chemical component of *Callemia Sinensis* (green tea) and many flavonoids including antibacterial activities of tea extracts and catchiness<sup>[4,5]</sup>.

The main objective of this research to screen and evaluate antibacterial activity of methanol extract of *Callemia Sinensis* and to find out zone of inhibition against both G+ve and G-ve bacteria.

### **Materials and Methods:**

#### **Plant Materials:**

The leaves of *Callemia Sinensis* were purchased from local market in Baghdad city.

The leaves of *Callemia Sinensis* (green tea) were cut into pieces, 25 gm of the leaves were soxhlet extracted using 350 milliliters of 95% methanol. The extraction tested for ten hours. The crude obtained was concentrated by evaporation using rotary evaporator at 100°C<sup>[6]</sup>.

#### **Microorganism:**

Test organisms were collected from Al-jerहत specialized hospital in Baghdad during the period of from 1/12/2008 to 1/7/2009: *Escherichia coli*, *Pseudomonas aeruginos* and *Proteus* species were isolated from urine samples of patients with urinary tract infections. *Streptococcus pneumoniae* were isolated from throat swabs of patients with tonsillitis. *Klebsiella*

*pneumoniae* were isolated from sputum of patients with upper respiratory tract infections. *Staphylococcus aureus* were isolated from patients with skin abscesses.

**Determination of zone of inhibition:**

Fifteen millimeters (15 ml) of sterile nutrient agar was poured into each sterile Petri dish of equal size and allowed to solidify. The surface of this sterile nutrient agar plate was streaked with pure culture of the standardized bacterial cell suspension. Acroborer (8 millimeter in diameter) was sterilized by flaming and used to create ditch at the center of the plate. The hole so created was then filled with the (50%) concentration of plant extract. The plates were allowed to stand for one hour for pre-diffusion of the extract <sup>[7]</sup> and incubation was done at 37 °C for 24 hours. At the end of incubation period, the diameter of zone of inhibition was measured in millimeters <sup>[8]</sup>.

**Results:**

The activity was evaluated using the agar diffusion method by measuring the diameter of the growth inhibition. Methanol extracts of *Callemia Sinensis* (green tea) plant used into traditional medicine of Iraq were submitted to a screening for antibacterial activity against six strains (table-1).

(Table-2) show the extracts obtained from *Callemia Sinensis* were found to be effective against *Escherichia coli* used in this study in different level.

(Table-3) show the extracts obtained from *Callemia Sinensis* were found to be effective against *Streptococcus pneumoniae*.

(Table-4) shows the methanolic extracts obtained from *Callemia Sinensis* were found to be effective against *Klebsiella pneumoniae*.

(Table-5) shows the methanolic extract obtained from *Callemia Sinensis* were found to be effective against *Staphylococcus aureus*.

(Table-6) show the methanolic extracts obtained from *Callemia Sinensis* were found to be effective against *Proteus* species.

(Table-7) show the methanolic extracts obtained from *Callemia Sinensis* were found to be effective against *Pseudomonas aeruginosa*.

No.	Species	No. of positive strains for antibacterial effect of <i>Callemia Sinensis</i> (green tea)
1	<i>E.coli</i>	8
2	<i>Str. Pneumoniae</i>	8
3	<i>Kleb. Pneumoniae</i>	12
4	<i>S.aureus</i>	8
5	<i>Proteus spp</i>	6
6	<i>Ps.aeruginosa</i>	12

**Table-1: Number of each bacterial species inhibited by antibacterial effect of *Callemia Sinensis* (green tea).**

Bacterial isolate	Ten fold dilutions of <i>Callemia Sinensis</i> extract											
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$	Cipro	
<i>E.coli</i>												
1	12	12	10	8	7	6	4	3	2	1	1	20
2	10	9	8	7	5	4	3	2	1	1	1	20
3	12	11	8	-ve	5	3	2	-ve	1	1	1	20
4	11	10	8	6	5	3	2	2	1	-ve	-ve	20
5	10	9	8	7	6	4	3	2	1	-ve	-ve	20
6	12	11	10	8	7	5	3	3	2	-ve	-ve	20
7	10	9	7	6	5	4	3	-ve	2	1	1	20
8	11	9	8	8	7	5	4	3	2	1	1	20

**Table 2: Zone of inhibition diameters of the ten fold dilutions of methanolic extract of *Callemia Sinensis* on the *E.coli* isolates compared with antibacterial effects of ciprofloxacin disc (5µg).**

Bacterial isolates	Dilutions(ten fold dilutions) of extract of <i>Callemia Sinensis</i>											
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$	Ci pro	
<i>Str.pneumone</i>												
1	11	11	10	9	7	6	5	2	1	1	1	27
2	15	14	12	10	9	8	5	4	3	2	2	27
3	12	10	10	9	7	6	4	3	2	1	1	27
4	12	11	9	7	6	5	4	2	1	1	1	27
5	13	11	8	8	7	-ve	5	3	2	1	1	27
6	12	11	10	9	8	7	6	5	4	2	2	27
7	13	12	9	8	7	7	-ve	5	3	2	2	27
8	12	10	8	6	5	4	3	2	1	1	1	27

**Table-3: Diameter of zone of inhibition (mm) of the methanol extract of *Callemia Sinensis* on the *Str.pneumoniae* isolates compared with antibacterial effects of ciprofloxacin disc (5µg).**

Bacterial isolates	Ten fold dilutions of <i>Callemia Sinensis</i> extract											
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$	cipro	
<i>Kleb. pneumoniae</i>												
1	13	11	9	8	7	6	5	4	5	6	27	
2	17	15	11	8	7	6	5	3	2	5	27	
3	15	14	12	10	6	4	-ve	-ve	4	4	27	
4	16	14	10	8	5	-ve	8	-ve	6	8	27	
5	20	17	11	9	7	5	4	-ve	5	5	27	
6	23	21	19	17	14	10	7	6	8	7	27	
7	22	22	20	18	16	13	8	6	8	8	27	
8	13	13	11	9	7	4	1	1	3	4	27	
9	14	11	9	9	7	6	3	3	5	4	27	
10	15	15	13	10	8	6	3	2	1	4	27	
11	13	11	9	8	8	4	8	6	7	7	27	
12	16	16	13	10	9	6	1	-ve	2	4	27	

**Table-4:** Diameter of zone of inhibition (mm) of the methanol extract of *Callemia Sinensis* on the *Kleb.pneumoniae* isolates compared with antibacterial effects of ciprofloxacin disc (5µg).

Bacterial isolates	Dilutions of <i>Callemia Sinensis</i> extract											
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$	Cipro	
<i>S.aureus</i>												
1	20	18	16	13	8	5	5	-ve	7	6	27	
2	18	17	15	11	9	7	-ve	-ve	3	5	27	
3	15	13	11	8	6	5	3	5	5	5	27	
4	20	19	16	12	9	5	3	-ve	8	5	27	
5	19	18	16	11	8	6	4	2	1	6	27	
6	15	14	12	11	10	9	7	5	4	1	27	
7	18	17	16	14	12	10	9	6	5	6	27	
8	18	16	15	13	11	-ve	-ve	5	4	2	27	

**Table-5:** Diameter of zone of inhibition (mm) of the methanol extract of *Callemia Sinensis* on the *S.aureus* isolates compared with antibacterial effects of ciprofloxacin disc (5µg).

Bacteria Isolates	Ten fold dilutions of <i>Callemia Sinensis</i> extract										
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$	Cipro
1	15	14	12	11	10	9	8	6	5	6	27
2	14	12	11	9	8	7	6	4	3	4	27
3	11	10	8	7	6	5	-ve	3	3	ve	27
4	12	11	9	8	7	6	5	4	3	-ve	27
5	15	14	13	11	8	7	5	4	3	3	27
6	14	13	11	10	8	6	-ve	5	3	5	27

**Table-6: Diameter of zone of inhibition (mm) of the methanol extract of *Callemia Sinensis* on the *Proteus spp* isolates compared with antibacterial effects of ciprofloxacin disc (5µg).**

Bacterial isolates	Dilutions(ten fold dilutions) of green tea extract										
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$	Cipro
1	17	17	12	-ve	6	6	3	2.5	3	4	27
2	15	13	13	13	8	-ve	3	2	2	3	27
3	12	12	11	8	-ve	8	4	3.5	2.5	2.5	27
4	9	7	-ve	7	5	4.5	3.5	3.5	4	5	27
5	10	10	10	8	7	6	3	4	3	4	27
6	10	8	8	7	7	7	-ve	4	4	-ve	27
7	11	11	11	10	9	6	4	5	3	4	27
8	12	10	10	10	9	8	6	4	4	6	25
9	10	9	9	9	8	5	4	4	5	7	25
10	17	15	15	12	8	7	5	4	4	5	25
11	15	13	11	10	10	8	7	5	6	-ve	25
12	13	12	12	7	6	6	4	4	3	6	25

**Table-7: Diameter of zone of inhibition (mm) of the methanol extract of *Callemia Sinensis* on the *Ps.aeruginosa* isolates compared with antibacterial effects of ciprofloxacin disc (5µg).**

### Discussion:

Green tea extends the effectiveness of sun screen and has been shown to have strong anti-irritant, anti-inflammatory and antibacterial properties for cleaner and healthier looking skin <sup>[9]</sup>. However, *Callemia Sinensis* have been to have potential chemo preventive activities. The extracts obtained from *Callemia Sinensis* (green

tea) were found to be effective against all tested microorganism used in this study at different level, showing several studies <sup>[10,11,12,13]</sup> have been conducted on the antibacterial properties of herbs spices and their derivatives such as essential oils, extracts and decoctions. In vitro studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. This result agreed with Hamilton and Otuke <sup>[14,15]</sup> who suggested that the antibacterial activity of *Callemia Sinensis* was properly due to their major component. The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Due to variable diffusability in agar medium, the antibacterial property may not demonstrate as zone inhibition commensurate to its efficacy.

All extracts were active against *E.coli* , *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus* species and *Pseudomonas aeruginosa*.

This result not agreed with Firas, <sup>[16]</sup> who reported that *Callemia Sinensis* had antibacterial activities against all pathogenic bacteria except *Proteus mirabilis*.

Ping *et al* 2008 <sup>[17]</sup> reported treatment with these agents may represent important adjuncts to, or alternative to, conventional antibiotic therapy.

Further evaluation of the antibacterial properties of these extracts and elucidation of the components responsible for the activities is warranted.

**Conclusions:** *Callemia Sinensis* have been shown to have potential chemotherapeutic activities. These data provide a direct mechanism of action for *Callemia Sinensis* and further support of its role as a chemopreventive agents.

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