



Photochemical and Antimicrobial Studies of Cinnamaldehyde and its Bioactive Derivatives

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trans-Cinnamaldehyde [3-phenyl-2-propanal] was isolated from essential oil of cinnamon (*Cinnamomum verum*). Schiff base and chalcone derivatives were synthesized through condensation reaction with acetone, acetophenone and aniline. Chalcone also subjected to photooxidation reaction in the presence of tetraphenylporphyrin (TTP) as singlet oxygen sensitizer leading to formation of endoperoxide through [2+4] cyclo addition. The endoperoxide undergo further breaking to form six diketone derivative. Antifungal and antibacterial studies were carried out on cinnamaldehyde and its derivatives i.e., chalcone, Schiff base and its dimer. Studies on the antifungal especially *Rhizoctonia solani* and *Penicillium italicum* and antibacterial activity against *Klebsiella pneumoniae* and methicillin resistant *Staphylococcus aureus* (MRSA) were showed that cinnamaldehyde and its derivatives have good antimicrobial.

Keywords: Photooxygenation, *trans*-Cinnamaldehyde, Chalcon, Tetraphenylporphyrin, *R. solani*, *P. italicum*, *S. aureus*.

INTRODUCTION

Plant extracts have been used for centuries for various purposes (traditional medicine, industrial applications, food preservatives) because of their antimicrobial properties and most of them are categorized under GRAS (generally recognized as safe) for human consumption¹. Many naturally occurring alkenylbenzene derivatives, usually relatively simple allyl- or propenylbenzene have been identified as components of various plants or their essential oils². Propenyl benzenes are often used as starting materials in the chemical synthesis of aroma compounds and fine chemicals. In the chemical industry, these are widely used as starting materials for synthesizing various products with applications as food preservatives and flavours. Although propenyl benzenes are usually toxic for most microbes, it has been reported recently they can be transformed into high-valued flavours by certain microorganisms³. α,β -Unsaturated aldehydes are important starting materials in various synthetic applications⁴. *trans*-Cinnamaldehyde [3-phenyl-2-propanal (**1a**)] is an aromatic aldehyde and main component of bark extract of cinnamon (*Cinnamomum verum*)⁵. It is present in essential oils of many plants and are proved to be active against many pathogenic bacteria^{6,7}, fungi⁶ and viruses⁸. Taking into account important activities of plant phenylpropenoids and their derivatives. Furthermore, phenylpropenoids were trapped the activated oxygen species *in vivo* to give the intermediated

endoperoxides and hydroperoxide derivatives, which could be alkylated or damage DNA, proteins and other biological species⁹⁻¹¹.

EXPERIMENTAL

***trans*-Cinnamaldehyde:** [3-Phenyl-propenal] (**1**) is the major component of essential oil of cinnamon which was extracted from *Cinnamomum verum*. IR spectra were performed on a Perkin-Elmer 16 FPC FT-IR spectrophotometer as thin films. ¹H NMR and ¹³C NMR spectra were obtained in CDCl₃ solution on a Bruker AVANCE D.P.X. 400 MHz apparatus. GCMS were determined by Joel JMS 600H, GC Hewlett Packerd, HP 6890 Series, with capillary column (30 m × 0.32 mm × 0.25 μ m) HP-5 cross linked 5 % dimethyl polysiloxane. A sodium lamp (Phillips G/5812 SON) was used for photo-irradiation reactions. Thin layer chromatography (TLC) and preparative layer chromatography (PLC): Polygram SIL G/W 254, Mecherey-Nagel. A rotatory evaporator (at 20 °C/15 torr) was used to remove the solvents.

Test organisms

Fungi pathogenic: *Rhizoctonia solani* and *Penicillium italicum* were obtained by the compilation of the Center for microbes (Mircen), Faculty of Agriculture, Ain Shams University-Arab Republic of Egypt. It was cultured on sabaroud dextros agar media (Oxoid CM 41) at 25 °C.

Rhizoctonia solani: It is the most important pathogen involved in cotton seedling disease¹² also rhizoctonia root and crown rot (RRCR) of sugar beet is caused by the soil borne fungus *Rhizoctonia solan* and it can affect potato plants from planting to harvest. It colonizes belowground potato plant surfaces in response to root and shoot exudates¹³.

Penicillium italicum: It is wound invading pathogens that causes decay on stored citrus fruits damaged by insects, animals, early splits, mechanical harvesting, chilling and environmental stresses. Green and blue mould caused by *Penicillium digitatum* and *P. italicum*, respectively, are the most important postharvest disease of fruits worldwide¹⁴.

Bacteria pathogenic: *Klebsiella pneumonia* and methicillin resistant *Staphylococcus aureus* (MRSA) from Laboratory of Jeddah King Fahad Hospital in Saudi Arabia. It were cultured on Mueller Hinton media (Oxioid CM 41) at 37 °C.

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the dangerous pathogenic bacteria that lived mainly in nasal membranes and skins of human or animals causing inflammations, blisters, sources as well as complication of acute wounds and burns resulting in inflammation of urinary tract, ear, eye and blood sepsis¹⁵.

Klebsiella pneumonia is an opportunistic pathogen that causes a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias and soft tissue infections¹⁶.

General photo-oxygenation of aromatic compounds

(4): A solution of **4** (10 mmol) in different solvents according to the type of sensitizers was irradiated externally by means of sodium lamp at -5 °C. During the irradiation a continuous stream of dry oxygen gas was allowed to pass through the reaction mixtures at a slow rate to avoid evaporation of solvent. The solvent was evaporated at 20 °C/15 Torr. The crude products were purified by column chromatography on silica gel adsorbent by eluting with a mixture of petroleum ether 60-80 °C and ether (9:2)².

1,5-Diphenyl-penta-2,4-dien-1-one (4): Yield (90%) needle yellow crystal, m.p. 100 °C, C₁₇H₁₄O (M 234.282). IR spectrum, $\nu_{\max}$, cm⁻¹: 3030, 2754, 1783, 1589, 1450, 1258 1005. ¹H NMR spectrum, δ (ppm): 7.035 t (1H, 4- H, *J* = 4.7 Hz) 7.10 d (1H, 5-H, *J* = 15 Hz), 7.34 d (1H-2-H, *J* = 7 Hz), 7.37-7.52 comp. pat. (5H, phenyl protons), 7.58 t (2 H, 3^l, 5^l-H, *J* = 8.12 Hz), 7.6 t (1H, 4^l-H, *J* = 4.7 Hz), 7.63 dd (1H, 3-H, *J* = 4.7Hz), 7.98 d (2H, 2^l, 6^l-H, *J* = 7 Hz). ¹³C NMR spectrum, δ (ppm): 125 (C⁴), 127 (C^{2l,6}), 127.5 (C²), 128.2 (C^{4l}), 128.4 (C^{3l,5l}), 129 (C⁵), 129.2 (C^{3l,5l}), 132.5 (C^{2l,6l}), 136 (C^{4l}), 138 (C^{1l}), 142 (C^{1l}), 145 (C³), 190 (C¹). Mass spectrum; *m/z* (I_{rel} %): 235 (10) [M⁺], 234 (100) [M⁺].

1,5-Diphenyl-penta-3-ene-1,2,5-trione (5): Yield (30 %) Colourless oil, C₁₇H₁₂O₃ (M 264.282). IR spectrum, $\nu_{\max}$, cm⁻¹: 2918, 1686, 1450, 1244, 1037. ¹H NMR spectrum, δ (ppm): 6.85 d (1H, 3- H, *J* = 4 Hz) 7.33 d (1H, 4-H, *J* = 4Hz), 7.46 t (3H, (3^l,4^l,5^l)-H, *J* = 18.15 Hz) H), 7.53 t (3H, (3^l, 4^l,5^l)-H, *J* = 7.15 Hz), 7.83 d (2H, 2^l, 6^l-H, *J* = 7 Hz), 8.01 d (2H, 2^l, 6^l-H, *J* = 7 Hz). ¹³C NMR spectrum, δ (ppm): 190 (C=O), 148 (C⁴), 145 (C³), 139 (C^{1l}phenyl), 137 (C^{1l} phenyl), 130 (C^{2l,6l,2l,6l}), 124 (C^{3l,5l,3l,5l}). GC-Mass data; retention time 21.52 min, *m/z* (I_{rel} %): 264, 282 (5) [M⁺], 234 (100) [M⁺-O₂],

159(20) (C₆H₆) [M⁺-COC₆H₅], 131 (20) [M⁺-C₂O₂C₆H₅], 97 (70) (C₆H₅).

Biological activity of cinnamaldehyde: Cinnamaldehyde and its derivatives were tested against the fungal species *Rhizoctonia solani* and *Penicillium italicum*, the bacterial species methicillin resistant *Staphylococcus aureus* and *Klebsiella pneumonia*.

Antifungal activities: Diffusion method was used to evaluate the antifungal activities of the tested compounds as follows: 1.0 and 0.5 mL of the tested compounds dissolved in chloroform (CHCl₃) (100.0 μ g mL⁻¹) were added to 50 mL of sabaroud dextrous agar media, then poured into sterile Petri dishes (9 cm in diameter) and left to solidify. Mycelia discs measuring 6 mm diameter were taken from the growing margins of cultures of *R. solani* and *P. italicum* (on SDA) and transferred on the surface in the middle of Petri dishes then incubated in 25 °C for 6 days in dark. The diameters of the fungal growth were measured after 2, 4 and 6 days¹⁷ (Tables 1 and 2).

Antibacterial activities

(a) 1 mL and 0.5 mL of the tested compounds dissolved in chloroform (CHCl₃) (100.0 μ g mL⁻¹) were added into nutrient broth media (Oxioid): The nutrient broth contained then 1 mL from suspension of methicillin resistant *Staphylococcus aureus* and *Klebsiella pneumonia* (10⁶ CFU/ μ L) at 37 °C for 24 h was added to it. The growth rate was measured monitoring change in optical density at 650 nm using a spectrophotometer after 24 h¹⁸ (Table-3).

(b) The agar disc diffusion method was employed for the determination of antibacterial activities **1a**, **2**, **3**, **4**, **5** and **6**¹⁹. Suspension of the tested microorganisms (10⁶ CFU/ μ L) was spread on Mueller Hinton Agar (Oxioid) for bacteria, filter paper discs (6 mm in diameter) were soaked with 20 μ L of the stock solutions and placed on the inoculated plates. After keeping at 2 °C for 2 h, they were incubated at 37 °C for 24 h. The diameter of the inhibition zones were measured in millimeters (Table-4).

Data analysis: Analysis of data was carried out by student's *t*-test for comparing the means of experimental and control groups²⁰.

RESULTS AND DISCUSSION

Cinnamaldehyde derivatives *i.e.*, phenyl-(3-phenyl-allylidene)-amine (Schiff base) (**2**), 1,9-diphenyl-nona-1,3,6,8-tetraen-5-one (**3**) and 1,5-diphenyl-penta-2,4-diene-1-one (chalcone) (**4**) were prepared by means of condensation of the respective carbonyl component in *trans*-cinnamaldehyde with aniline²¹, acetone and acetophenone²² respectively (**Scheme-I**).

Interestingly, the photo induced oxygenation of compounds **4** in the presence of tetraphenylporphyrin (TPP) as singlet oxygen sensitizer led to the formation of 1,5-diphenylpent-3-ene-1,2,5-trione (**5**) as a sole photo-products.

The structure of compound **5** was established by spectral measurements. IR spectrum contained an absorption band at 1686 cm⁻¹ for carbonyl group, which is shifted from 1783 cm⁻¹ in the starting material. ¹H NMR spectrum of **5** showed doublet at δ 6.85 ppm and δ 7.33 ppm from protons on C³ and C⁴ respectively, as triplet at δ 7.46 and at δ 7.53 ppm for protons

TABLE-1
EFFECT OF DIFFERENT CONCENTRATIONS OF **1a**, **2**, **3**, **4**, **5** AND **6** ON THE RADIAL GROWTH AND INHIBITION OF *Penicillium italicum* GROWN ON SOLID MEDIA (mm/disc; MEAN OF REPLICATES $\pm$ SE)

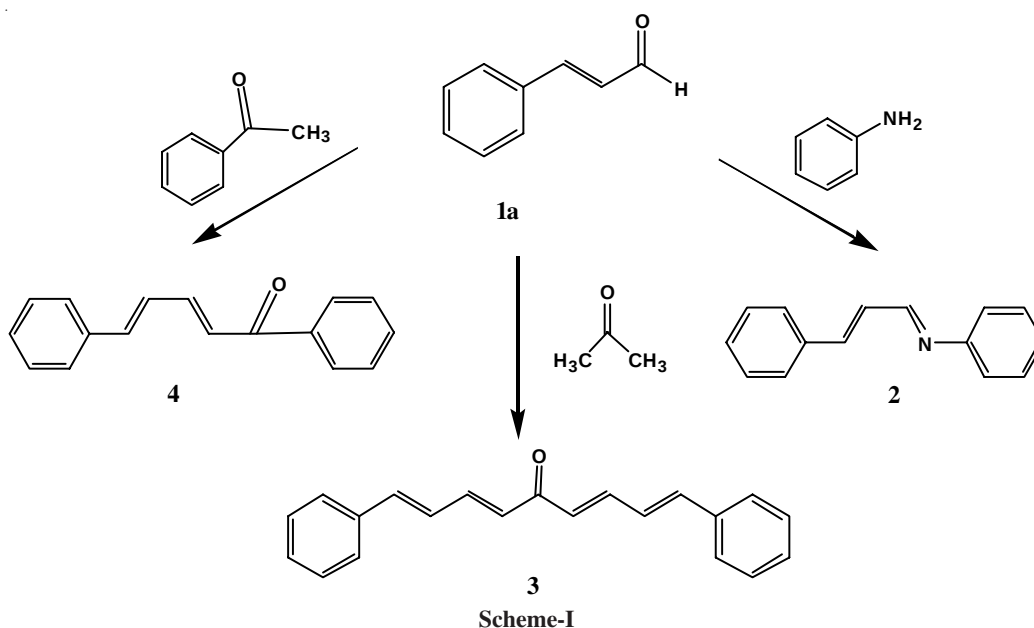
Treatment	Concentration % (ppm)	Incubation (days)					
		2		4		6	
		Radial growth	Inhibition (%)	Radial growth	Inhibition (%)	Radial growth	Inhibition (%)
Control	0.0	3.533 $\pm$ 1.584	0.00	6.117 $\pm$ 1.82	0.00	8.532 $\pm$ 2.012	0.00
1a	0.5	2.433 $\pm$ 1.548**	31.135	3.617 $\pm$ 1.619**	40.869	4.150 $\pm$ 1.857**	51.359
	1.0	1.633 $\pm$ 0.733	53.778	2.217 $\pm$ 1.000	63.756	2.833 $\pm$ 1.070	66.795
2	0.5	1.617 $\pm$ 0.726*	54.231	3.167 $\pm$ 1.424*	48.226	6.333 $\pm$ 1.49*	25.773
	1.0	0.667 $\pm$ 0.333**	81.120	2.233 $\pm$ 0.567	63.495	4.667 $\pm$ 0.760**	45.300
3	0.5	2.667 $\pm$ 1.194	24.511	3.583 $\pm$ 1.603*	41.425	3.567 $\pm$ 1.596*	58.192
	1.0	2.633 $\pm$ 0.733**	56.956	1.450 $\pm$ 0.752	58.958	2.583 $\pm$ 1.156	69.725
4	0.5	0.533 $\pm$ 0.120	84.913	3.517 $\pm$ 1.577	42.504	6.883 $\pm$ 1.871	19.327
	1.0	1.633 $\pm$ 0.924	53.778	3.017 $\pm$ 1.349	50.678	5.383 $\pm$ 1.517	36.908
5	0.5	0.967 $\pm$ 0.433**	72.629	2.088 $\pm$ 1.300**	65.865	3.000 $\pm$ 1.165	64.838
	1.0	0.467 $\pm$ 0.211	86.781	1.767 $\pm$ 0.819	71.113	1.990 $\pm$ 0.651	76.676
6	0.5	1.239 $\pm$ 342	64.930	2.890 $\pm$ 0.009**	52.754	3.640 $\pm$ 0.432**	57.337
	1.0	0.882 $\pm$ 411	75.035	1.567 $\pm$ 2.061	74.382	2.000 $\pm$ 0.213	76.558

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; (ns)Non-significant at $P \leq 0.05$

TABLE-2
EFFECT OF DIFFERENT CONCENTRATIONS OF **1a**, **2**, **3**, **4**, **5** AND **6** ON THE RADIAL GROWTH AND INHIBITION OF *Rhizoctonia solani* GROWN ON SOLID MEDIA (mm/disc; MEAN OF REPLICATES $\pm$ SE)

Treatment	Concentration % (ppm)	Incubation (days)					
		2		4		6	
		Radial growth	Inhibition (%)	Radial growth	Inhibition (%)	Radial growth	Inhibition (%)
Control	0.0	6.667 $\pm$ 0.105	0.00	7.667 $\pm$ 0.214	0.00	9.000 $\pm$ 000	0.00
1a	0.5	4.833 $\pm$ 0.173	27.508	6.467 $\pm$ 0.165*	15.651	7.133 $\pm$ 0.042	20.740
	1.0	3.167 $\pm$ 0.105**	52.497	4.100 $\pm$ 0.037**	46.524	4.200 $\pm$ 0.036**	53.333
2	0.5	3.600 $\pm$ 0.132**	46.002	4.233 $\pm$ 0.092	44.789	5.700 $\pm$ 0.079**	36.660
	1.0	1.367 $\pm$ 0.184	79.496	1.833 $\pm$ 0.069	76.092	5.133 $\pm$ 0.042	42.966
3	0.5	5.967 $\pm$ 0.184**	10.499	6.333 $\pm$ 0.152**	17.399	6.900 $\pm$ 0.037	23.333
	1.0	3.233 $\pm$ 0.092*	51.504	3.967 $\pm$ 0.021*	48.258	3.213 $\pm$ 0.542*	64.300
4	0.5	4.378 $\pm$ 0.006	34.333	5.871 $\pm$ 0.0567	23.425	7.443 $\pm$ 0.321	17.300
	1.0	3.567 $\pm$ 0.124	46.497	3.984 $\pm$ 1.543	48.037	6.231 $\pm$ 0.008	30.766
5	0.5	1.987 $\pm$ 0.098	70.196	3.456 $\pm$ 0.054	54.923	4.009 $\pm$ 3.206	55.455
	1.0	0.892 $\pm$ 0.032	86.620	1.561 $\pm$ 0.761	79.640	2.002 $\pm$ 0.014	77.755
6	0.5	2.561 $\pm$ 0.008	61.586	4.303 $\pm$ 0.002	43.876	5.552 $\pm$ 0.986	38.311
	1.0	1.330 $\pm$ 0.012	80.050	2.223 $\pm$ 1.125	71.005	2.133 $\pm$ 0.042	76.300

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; (ns)Non-significant at $P \leq 0.05$

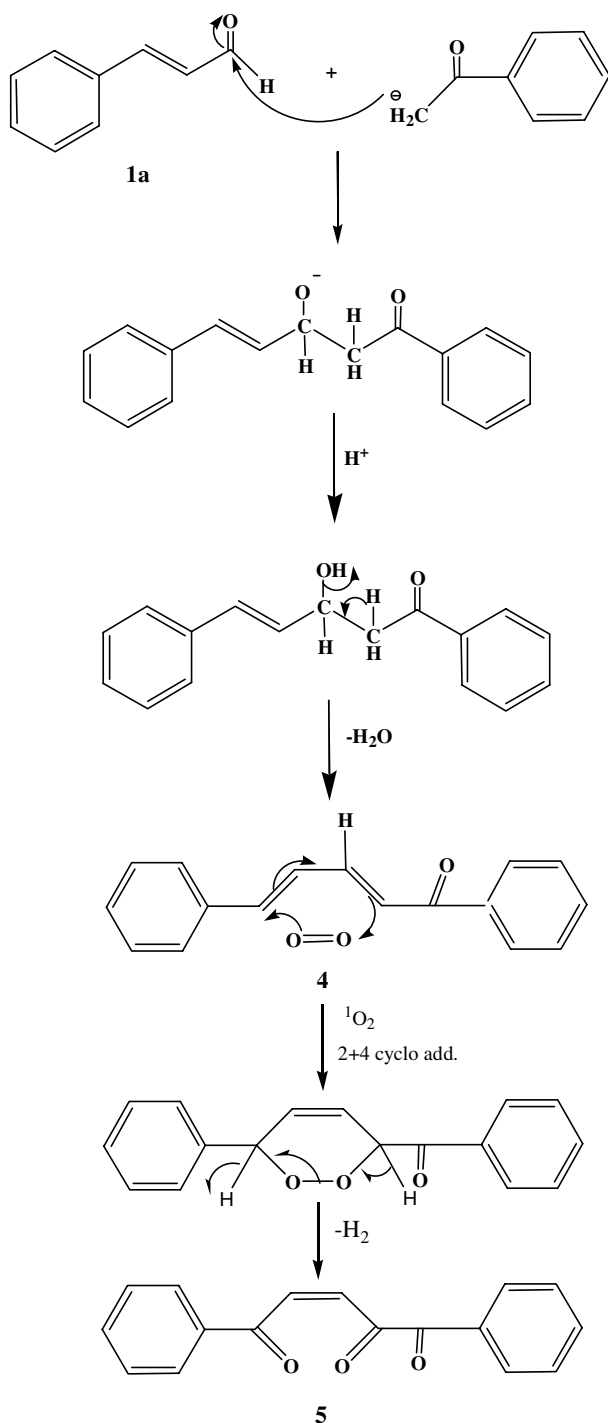


on $C^{3\text{I}}, 4\text{I}, 5\text{I}$ and $C^{3\text{II}}, 4\text{II}, 5\text{II}$, respectively. Doublet at δ 7.83 and at δ 8.01 ppm for protons on $C^{2\text{I}}, 6\text{I}$ and $C^{2\text{II}}, 6\text{II}$ respectively.

^{13}C NMR spectrum of compound **5**, the $C^{1\text{I}}$ phenyl and $C^{1\text{II}}$ phenyl signals were located at δ_{C} 137 and 139 ppm respectively. C^3 appeared at δ_{C} 145 ppm whereas C^4 at δ_{C} 148 ppm.

The $C^{3\text{I}}, 5\text{I}, 3\text{II}, 5\text{II}$ and $C^{2\text{I}}, 6\text{I}, 2\text{II}, 6\text{II}$ were located at δ_{C} 124 ppm and δ_{C} 130 ppm resonated respectively. Signals at δ_{C} 189 ppm for three carbonyl groups.

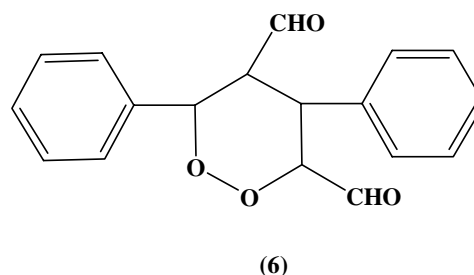
Scheme-II illustrates a probable mechanism of the photosensitized oxygenation of 1,5-diphenyl-penta-2,4-diene-1-one (**4**) in the presence of tetraphenylporphin leading to formation of endoperoxide through [2+4] cyclo addition. The



Scheme-II

endoperoxide undergo further breaking to form diketone (**5**), the endoperoxide intermediate could not be isolated.

Dimeric cinnamaldehyde (4,6-diphenyl-1,2-dioxane-3,5-dicarboxaldehyde) (**6**) has been photosynthesis. It was strongly inhibited *Candida albicans* growth more than its monomer²³.



Fungal and bacterial diseases are among the most common infections. The treatment is limited, for many reasons and new drugs are necessary. In this work, the antimicrobial activity against *Penicillium italicum*, *Rhizoctonia solani*, *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae* were also studied, and the results obtained showed the important antimicrobial activity of cinnamaldehyde and its derivatives (Tables 1-4). The antifungal result (Tables 1 and 2) showed that the growth of *P. italicum* and *R. solani* on the solid media were reduced in the presence of cinnamaldehyde and its derivatives on fungi. The data in (Table-3) revealed that there were a significant decrease in the growth of *S. aureus* and *K. pneumoniae* on liquid media, when they were treated by cinnamaldehyde and its derivatives. The same result has been explained in the diffusion method which showed inhibition zones around the filter paper discs (Fig. 1). If the inhibition zone measures 2 and 3 mm, then the complex has a good antibacterial action. If the inhibition zone measures more than 3 mm across, then it is considered very effective but if there is no inhibition zone then the complex has no activity on the bacterial growth and will not be retained for treatment²⁴. It is clear from Table-4, the emergence of the inhibition of the growth rate of pathogenic bacteria *S. aureus* and *K. pneumonia* as a result of various transactions, with 2,4,5,7, 7-1 and dimer. It was found that when the transaction intended to 7-1 report inhibition zone formed around the filter papers saturated with about 18.22 ± 0.00 and 12.02 ± 0.014 mm respectively, while appearance lesser extent of the inhibition zone of bacteria of the dimer reaching 3.21 ± 40.0 and 5.42 ± 221 mm for each of the *S. aureus* and *K. pneumonia*.

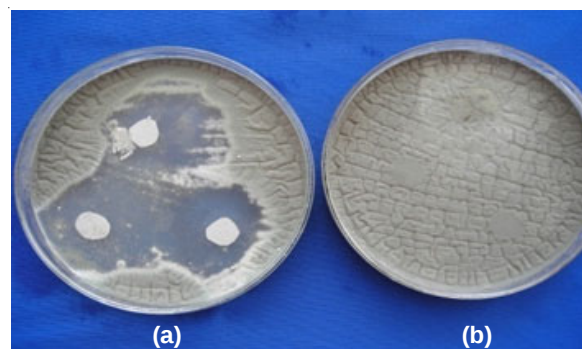


Fig. 1. Effect of 1,5-diphenyl-penta-3-ene-1,2,5-trione (**5**) on radial growth of fungi grown on the solid media (a) *Penicillium italicum* (b) control

TABLE-3
EFFECT OF VARIOUS CONCENTRATIONS OF **1a**, **2**, **3**, **4**, **5** AND **6** ON THE DRY WEIGHT OF
Staphylococcus aureus AND *Klebsiella pneumoniae* AFTER 24 h (MEAN OF REPLICATES $\pm$ SE)

Treatment	Concentration (ppm)	<i>Staphylococcus aureus</i>		<i>Klebsiella pneumoniae</i>	
		Growth rate	Inhibition (%)	Growth rate	Inhibition (%)
Control	0.0	85.000 $\pm$ 1.826	0.0	90.000 $\pm$ 0.966	0.0
1a	0.5	30.333 $\pm$ 0.211**	64.70	45.020 $\pm$ 0.365	49.97
	1.0	22.533 $\pm$ 0.201**	74.11	24.867 $\pm$ 0.128**	72.37
2	0.5	64.733 $\pm$ 0.015*	23.84	56.022 $\pm$ 1.142*	37.75
	1.0	41.667 $\pm$ 0.201	51.23	43.100 $\pm$ 0.030	52.11
3	0.5	70.000 $\pm$ 0.121**	17.64	61.303 $\pm$ 0.008**	31.88
	1.0	35.000 $\pm$ 0.004*	58.82	40.700 $\pm$ 0.212*	54.77
4	0.5	77.000 $\pm$ 0.001	9.41	68.051 $\pm$ 0.112	46.61
	1.0	57.331 $\pm$ 0.242	32.55	53.114 $\pm$ 0.271	40.98
5	0.5	23.124 $\pm$ 0.002	72.79	25.561 $\pm$ 0.200**	71.59
	1.0	16.223 $\pm$ 0.110	80.91	14.942 $\pm$ 0.001**	83.39
6	0.5	76.013 $\pm$ 0.540	10.57	65.020 $\pm$ 0.401	27.75
	1.0	65.200 $\pm$ 0.081	23.29	58.223 $\pm$ 0.400	35.30

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; (ns)Non-significant at $P \leq 0.05$

It has been concluded that *trans*-cinnamaldehyde and its derivatives can act as antioxidants. They were trapped the reactive oxygen species (ROS) to give the intermediated epoxides and hydroperoxide derivatives, which could be alkylated or damage DNA, proteins and other biological species.

From the above result cinnamaldehyde and its derivatives show high activity against the growth of bacteria and fungi, but the inhibition was in bacteria more than of fungi, this may be explained on the basis bacteria is prokaryotic but fungi are eukaryotic and the sensitivity of prokaryotic is different than that of eukaryotic because the changes in the cell wall and plasma membrane and also the nuclear substances moreover. Cinnamaldehyde and its derivatives can act as fungicidal and bactericidal agents. The results revealed that the inhibitory effect of **5** on rate growth of *P. italicum* was more effect than *R. solani*.

Furthermore the cinnamaldehyde and its derivatives yield clear inhibition zones around the filter paper discs. These showed their activity against on Gram-positive bacteria methicillin resistant *S. aureus* and negative bacteria *K. pneumoniae*, but **5** against showed more inhibitory effect on *S. aureus* than *K. pneumoniae*.

Conclusion

Studies on the antifungal especially *Rhizoctonia solani* and *Penicillium italicum* and antibacterial activity against the *Klebsiella pneumoniae* and methicillin resistant *Staphylococcus aureus* (MRSA) were showed that cinnamaldehyde and its derivatives have a good antimicrobial activity.

REFERENCES

- M. Busquet, S. Calsamiglia, A. Ferret, P. Cardozo and C. Kamel, *J. Dairy Sci.*, **88**, 2508 (2005).
- E. Elgendy and S. Khayyat, *Russ. J. Org. Chem.*, **44**, 823 (2008).
- P. Xu, D. Hua and C. Ma, *Trends Biotechnol.*, **25**, 571 (2007).
- N. Nordqvist, C. Björkelid, M. Andaloussi, A. Jansson, S. Mowbray, A. Karlén and M. Larhed, *J. Org. Chem.*, **76**, 8986 (2011).
- S. Ali, A.A. Khan, I. Ahmed, M. Musaddiq, K.S. Ahmed, H. Polasa, L.V. Rao, C.M. Habibullah, L.A. Sechi and N. Ahmed, *Ann. Clin. Microbiol. Antimicrob.*, **4**, 20 (2005).
- N.S. Shashidar, Ph.D. Thesis, Studies on Bioactive Natural Compounds for Their Antimicrobial and Antioxidant Properties, Department of Microbiology, Osmania University, Hyderabad, India (2002).
- P. Suresh, V. Ingle and V. Vijaya, *J. Food Sci. Technol.*, **29**, 254 (1992).
- P. Pacheco, J. Sierra, G. Schmeda-Hirschmann, C.W. Potter, B.M. Jones and M. Moshref, *Phytother. Res.*, **7**, 415 (1993).
- E. Elgendy and S. Khayyat, *Russ. J. Org. Chem.*, **44**, 814 (2008).
- A.A. Saddiq and S. Khayyat, *Pesticide Biochem. Physiol.*, **98**, 89 (2010).
- E.M. Elgendy and S. Khayyat, *Asian J. Chem.*, **26**, 6571 (2014).
- C.S. Rothrock, in eds.: B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst, Cotton Diseases incited by *Rhizoctonia solani*, In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control (1996).
- M.J. Jeger, G.A. Hide, P.H.J.F. Van Den Boogert, A.J. Termorshuizen and P. Van Baarlen, *Potato Res.*, **39**, 437 (1996).
- M.S. Ladaniya, Citrus Fruit: Biology, Technology and Evaluation, Academic Press, USA, pp. 594 (2008).
- L. Chateau, Alternatives to Antibiotics from Nature, Jarir Bookstore, Saudi Arabia, edn 2, pp. 29-36 (2004).
- R. Podschun and U. Ullmann, *Clin. Microbiol. Rev.*, **11**, 589 (1998).
- G.L. Bollen, *J. Plant Pathol.*, **78**, 5 (1972).
- D. Stormonth and G. Coleman, *J. Gen. Microbiol.*, **71**, 407 (1972). (rgp).
- Hasenekoglu, Laboratory Techniques for Micro fungi", Atatürk University, Erzurum, Turkey, pp. 66, 1990.
- H.B. Hill, Principles of Medical Statistic, Oxford University, edn 9 (1971).
- A.I. Vogel, Elementary Practical Organic Chemistry, edn 2, vol. 1, William Clowes & Sons, Great Britain (1975).
- H. Muathen, M. Abou-Elzahab and K. Qutub, *Aust. J. Bas. Appl. Sci.*, **1**, 586 (2007).
- S.A. Khayyat, *J. Saudi Chem. Soc.*, **17**, 61 (2013).
- D. Baudoux, Antiviral and Antimicrobial Properties of Essential Oils (1991); Available from <http://www.aromabar.com/articles/naud55.htm>.