

ANTIBACTERIAL EFFICIENCY AND DNA IMPAIRMENT UNVEIL IN SOME BACTERIA
STRAINS TREATED WITH *CONOCARPUS ERECTUS* L. EXTRACT

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ABSTRACT: *Conocarpus erectus* L. (Family *Combretaceae*) is an introduced plant species, widely spread in Jeddah City, and other parts of Saudi Arabia. Folk medicine has used Parts of this species in its native countries. In the present work four different concentrations of ethanol extracts (50; 100; 150 & 200ppm) from different parts of this plant (seeds, stem, leaves and bark) were assessed against three Gram+ve bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*) and three Gram-ve bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* & *Klebsiella pneumoniae*). The effect magnitude of these extracts on tested bacteria strains was as follows: leaves extracts bark stem seeds. Nevertheless results showed a strong activity of all these different parts against *Staphylococcus aureus* among the Gram+ve bacteria. The maximum inhibitory effect was observed at 200 μ L /dish by all used parts extracts except the bark extract against *Pseudomonas aeruginosa*, followed by *Escherichia coli* and *Klebsiella pneumoniae* among the Gram-Ve bacteria. The Random Amplified Polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in treated bacteria strains. The results confirmed the potentials of *Conocarpus erectus* as good antibacterial source. Worth noting that the present report, is the first on the antibacterial and molecular characterization of bacteria strains by *Conocarpus erectus*, growing in the west coast of Saudi Arabia. In conclusion we recommend more innovative investigations, in order to explore the treasures of this therapeutically important plant species.

Key words: Antibacterial efficacy, DNA, Bacteria, *Conocarpus erectus* L

INTRODUCTION

Bacterial infections causes high rate of mortality among human population and aquaculture organisms (Kand hasamy and Arunachalam, 2008). Due to this and because of the increasing in discriminate use of antibiotics, pathogenic bacteria becoming resistant to drugs (Lavanya and Veerappan, 2011). Natural alternatives with desirable side effects are therefore needed (Ushimaru et al., 2007). Such effective and save antibiotics Known to be derived from plant species, in sufficient quantities and quality (Urszula et al., 2010). Different plant parts are used medically eg, rhizomes, bulbs, leaves ,root, barks and peels (Anne- Catherine,2007).Medicinal plants are the richest bio-resource for drugs used in traditional and modern medicines; nutraceuticals; food supplementsm ; pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al,2008; Nirmala et al.,2011). *Conocarpus erectus* Lis one of two species in the genus concarpus, in the family Combretaceae growing on low slopes and shorelines in tropical and subtropical regions around the world (Bailey, 1976). *Conocarpus erectus* L used in its native countries as folk remedy for anemia, catarrh, conjunctivitis, diabetes, diarrhea and fever (Irvin, 1961). *C.erectus* is widely spread in Jeddah city and other parts of Saudi Arabia. RAPD-PCR considered as one of the most reliably used techniques in the dedication of DNA damages. These damages are most probably caused by genotoxic treatments. Such damages occur in RAPD consequential to DNA impairment, including gains or losses of bands, structural re-arrangements, variation in band intensity (Atienzar et al.,2002).The RAPD method is increasingly applied to detect genetic instability and successfully detected genomic DNA alterations induced by several DNA affecting agents (Papadopoulos et al.,2002). In the present study RAPD test was utilized. In six strains of bacteria exposed to the ethanol extracts of different parts of *Conocarpus erectus* L.

MATERIALS AND METHODS

Samples collection and preparation

Plants materials collection and preparation

The different parts (leaves, seeds, bark and stems) of the plant under investigation were collected during September -2012 from Jeddah city, (21°24'3"N;39°17'45"E) Saudi Arabia.

Species status of this plant was fervid at Faculty of Sciences Herbarium, King Abdulaziz University, Jeddah. The samples were brought to the laboratory, thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water for five minutes, then air-dried under room temperature until constant weight.

Extracts preparation

Ten grams of dried *Conocarpus erectus* parts (leaves, seeds, bark and stems) were cut into small pieces by blender 1-2 mm. Extraction was done by adding 100 ml of ethanol (1:10W/V) left under cold conditions for 48h. then the extracts were filtered through a filter paper. The extracts solutions were evaporated under reduced pressure at 40°C until dryness, subsequently, the extract was diluted by dimethyl sulf.oxide (DMSO) and stored under 20°C until analysis according to (Boeru and Derevici, 1978).

Bacterial strains

Cultures were prepared for in vitro antibacterial assay of the six bacteria strains, three Gram positive: *Bacillus subtilis* (ATCC11774); *S. aureus* (ATCC29213) and *Micrococcus luteus* (ATCC4698) and three Gram negative *Escherichia coli* (ATCC8739); *Klebsiella pneumoniae* (ATCC700603) and *Pseudomonas aeruginosa*. (ATCC27853)

Those strains were provided by Microbiologics® USA. Tested organisms were sub cultured on nutrient agar (Oxoid laboratories, UK) slopes. These stock cultures were stored in the dark at 4°C until used.

Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce (1994). DMSO was used as a negative control. The plates were done in triplicate. Bacterial cultures were incubated at 37°C for 24 h at. Antimicrobial activity was determined by measurement zone of inhibition (Agwa et al., 2000).

DNA extraction and RAPD – PCR amplification conditions.

Genomic DNA was prepared from 18h cultures in exponential phase in Luria – Bertani medium (1000 mL deionized water ,10 g Bactotryptone, 5 g Bacto yeast, 5 g NaCl several drops, 5 M NaOH several drops and 1 M HCl) . Aliquots of 10 ml of each bacterial culture were harvested by centrifugation at 12,000 rpm for 15 min at 4 °c and washed once in sterile distilled water. The pellets were resuspended in 400 µl of lysis buffer containing 2% glucose, 50 mM Tris – HCl(pH 8.0), 25 mM EDTA, 3 mg / ml lysozyme and 200mg / ml RNase. The cell suspension was incubated for 1h at 37°C. Further DNA extraction was performed as described by Sambrook *et al.* (1989). PCR amplification was carried out in a DNA thermocycler (Biomatra, Germany) for 30 cycles each. The PCR reaction was carried out in a final volume of 25 µL with 1X PCR buffer containing 10 mM Tris – HCl, 25 mM MgCl₂, 1 ml of Template DNA, 0.2 mM deoxy nucleoside triphosphate, 1 to 2 µM (each) primer and 0.5 u of Taq DNA polymerase (Promega). PCR conditions consisted of initial denaturation at 95°C for 2min followed by 95°C for 1min, annealing to primers at 37°C for 1 min, and extension at 72°C for 1 min with a final extension step at 72°C for 5 min. PCR – amplified products were separated using agarose gel electrophoresis in 1% TBE buffer (Tris base 108 g, boric acid 55 g, 0.5M EDTA 20 ml and H₂O₂ to 1 L) dilute accordingly for 1X stock and stained with 0.2 µg /ml ethidium bromide according to Sambrook *et al.* (1989). Amplified fragments were detected and photographed under UV light. Two random primers were synthesized from University of British Columbia (UBC), Vancouver, Canada (UBC 16 , 28 and 89) and their sequences are presented in Tabulation. The random primer names and sequences that used for RAPD analysis. Williams *et al.* (1990).

Primer	Sequences
Primer A7	G AAACGGG TG
Primer B5	TGCGCCCTTC

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activity of ethanolic extracts of seeds ,stem, leaves and bark of *C. erectus* was assessed against three Gram +ve bacteria (*B.subtilis*, *M.lutues* and *S.aureus*) , three Gram – ve bacteria (*E.coli*, *K.pneumonia* and *P. aeruginosa*) at different concentrations ranged from (50-200 µL), the leaves extract showed the highest activity against tested organisms followed by the bark > stem> seeds respectively (Table 1).It can be deduced that *Conocarpus erectus* leaf ethanol extract had the broadest spectrum of activity on the tested bacteria.

The magnitude of its inhibition zones against the tested bacteria was as follows: *S. aureus* (36.7mm), *B. subtilis* (26.7mm), *M. luteus* (25.7 mm), *P. aeruginosa* (35.7mm), *E. coli* (24.0 mm) and *K. pneumoniae* (22.7 mm) were more sensitive at 200 µl /plates concentration. *Conocarpus erectus* bark extract exhibited remarkable antibacterial activities against the tested bacteria in the following sensitivity order *S. aureus* > *P. aeruginosa* with equal effect on *K. pneumoniae* and *B. subtilis* but the bark extract's antibacterial action showed the least effect on *E. coli* (22mm) at concentration of 200µl/plates. The stem extract showed the highest activities against *S. aureus* and *P. aeruginosa* (32.3 and 32.0) then on *K. pneumoniae* (21.00) at concentration of 200µl. Seeds extract have the least antibacterial activity compared to other parts. The present results coincided with Bakar, et al. (2010) whom reported that *Moringaolifera* seeds chloroform extract have the least effect on the tested organisms. They also stated that *Moringaolifera* seeds MSC extract low antibacterial activity might be linked to their phytochemical contents, due to that alkaloids, tannins and flavonoids were not detected in MSC extract of those seeds. Presence of such compounds in plant species parts enhances their antimicrobial properties Singh and Bhat (2003) and Tschache (1971). In previous report on *Conocarpus erectus*, the methanol extract of leaves, stem, fruits, and flowers showed antimicrobial properties (El-Sayed et al., 2012). However their reported inhibition zones were far less than those recorded in the present study, eg. records of leaves and stems extracts. This may indicate an ecotype differences in this plant species, hence the currently studied plant grows under higher temperature and drier climate. Such circumstances increase the concentrations of the active substances in the plant parts, and thereby their effects on the tested organisms. Phenolic compounds, especially tannins are the major components of this plant species (El.Sayed et al., 2012). This would suggest that the antimicrobial effects observed in this study and with more pronounced effects in present study could be attributed to such compounds. Therefore it would be of great benefit to separate those compounds and use them on the same bacteria strains, in order to verify the usefulness of this important plant species. Moreover to produce more abundant and inexpensive natural antibiotics. Gumgumjee et al. (2012) stated that *Tamarindus indica* leaves ethanol extract has produced strong antibacterial effects against *S. aureus* > MRSA > *B. subtilis* respectively. *S. aureus* showed also similar results when treated with *Casuarina equisetifolia* leaves ethanol extract (Gumgumjee and Hajar, 2012). The higher sensitivity of Gram +ve bacteria to natural plants extracts has been documented (Dulger and Gonuz, 2004; Chaieb et al., 2011). The ethanol extracts of different parts of *Conocarpus erectus* were also tested on Gram – ve bacteria; *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The results showed a strong effect against *P. aeruginosa* followed by *E. coli* and *K. pneumoniae* at 200 µl /dish by all tested parts of *Conocarpus erectus* bark extracts. The present results agreed to some extent with El-Sayed et al. (2012). The susceptibility of bacteria strains to plant species extracts may draw attention to plants potentials as a natural antibiotic producers, these can be used against the susceptible bacteria strains (Khosravi & Behzadi, 2006). Due to the nature of the bacteria species response, differences between them are expected (Moyo et al., 2011). Results obtained from present study provide more evidence on the antimicrobial efficacy of the different plant parts, eg. leaves, bark, stem and seeds although they differ in their magnitude. The mechanisms of actions of these compounds have been proven to be via cell membranes perturbations (Esimone et al., 2006).

Molecular characterization of Bacteria by RAPD analysis

Two random primers A7 and B5 were used for RAPD analysis of the tested bacteria.

RAPD analysis of Gram +ve bacteria using A7 random primer

Primer A7 with *B. subtilis* revealed 13 fragments were polymorphic with size ranged from 5772.895 to 176.428Kb and 2 fragments were common (Fig 1 and Table 2.A). The total band number of the treated bacteria was higher than the control (9 Vs .6). It is interesting to note that 9 fragments were uniquely displayed in the treated *B. subtilis* ATTC11774 with 100 µl ethanol extract of *Conocarpus erectus*, while other 4 fragments were detected in the control and absent in the treated bacteria. Both of the two fragments types clearly showed the effect of the treatment. Primer A7 with *S. aureus* ATTC29213 revealed 8 fragments were common (Fig 1 and Table 2.A). The total band number of the treated sample was closely equal to the control (7 vs.6). It is interesting to note that 2 fragments were uniquely displayed in the treated *S. aureus* with 100 µl Ethanolic extract of *Conocarpus erectus*. However, 1 fragment was uniquely displayed in the control and disappeared in the treated sample. Therefore, both two types of fragments clearly showed the effects of the treatment. Primer A7 with *M. luteus* ATTC 4698 revealed 7 fragments, whereas 5 fragments were polymorphic with sizes ranged from 2528.518 to 162.4 Kb and 2 fragments were common (Fig 1 and Table 2.A). The total band number of the treated sample was closely near to the control (5 vs.4). However, 3 fragments were uniquely displayed in the treatment sample. While other two fragments were detected in the control and displayed in the treatment sample. Therefore, both fragments clearly showed the effects of the treatment, where the three fragments were induced under treatment and the two other fragments were disappeared and replaced by the other three.

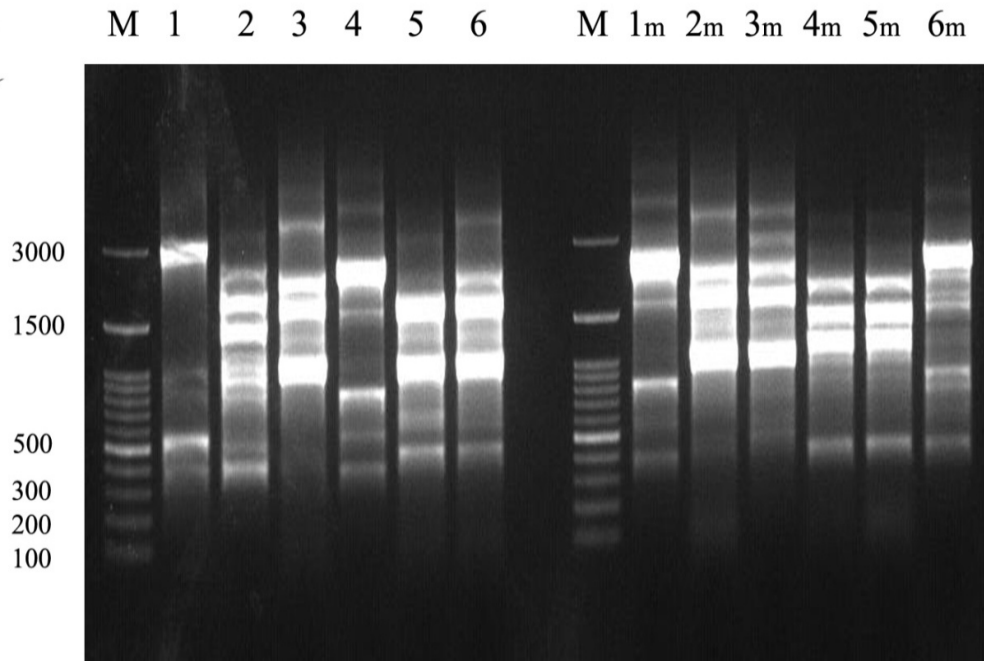


Figure 1. RAPD patterns for 6 strains of Bacteria obtained with A7 random primers :1, control *B.s* ATCC 11774; 2, control *S.aureus* ATCC 29213; 3, control *M.luteus* ATCC 4698 ; 4,, control *P.aeruginosa* ATCC 27853; 5, *K.Pneumonia* ATCC 700603 ; 6, *E.coli* ATCC 8739; 1m, Treated *B.s*; 2m, Treated *S.aureus*; 3m, Treated *M.luteus*; 4m, Treated *P.aeruginosa*; 5m, Treated *K.Pneumonia*; 6m, *E.coli*

Table 1. Zones of inhibition of ethanol extracts of different parts of *Conocarpus erectus* against different types of bacteria.

Part Used		Gram positive bacteria			Gram negative bacteria			Means of Bacteria
Leaves	Concentrations	<i>B.subtilis</i> A TCC11774	<i>M.lutues</i> ATCC4698	<i>S.aureus</i> ATCC29213	<i>E.coli</i> ATCC8739	<i>K.pneumonia</i> ATCC700603	<i>P.aeruginosa</i> ATCC27853	
Seeds	50	21.67±0.33	19.67±0.33	31.67±0.88	19.00±0.58	20.00±0.00	28.67±0.33	23.45
	100	24.00±0.58	21.67±0.33	34.67±0.33	20.76±0.33	20.76±0.33	32.67±0.33	25.75
	150	25.00±0.00	23.00±0.00	35.67±0.33	22.67±0.33	21.33±0.33	34.33±0.33	27.00
	200	26.76±0.33	25.67±0.33	36.67±0.33	24.00±0.00	22.67±0.33	35.67±0.33	28.57
	50	18.33±0.33	16.67±0.33	23.33±0.33	00.00±0.00	14.67±0.33	00.00±0.00	18.25
	100	19.76±0.33	18.00±0.58	24.67±0.33	16.33±0.33	15.67±0.33	00.00±0.00	81.89
	150	21.00±0.58	19.33±0.33	26.33±0.33	17.67±0.33	16.00±0.58	24.00±0.58	20.72
	200	22.00±0.58	23.33±0.33	30.67±0.33	21.67±0.33	16.33±0.33	27.67±0.33	23.61
Park	50	19.00±0.58	18.00±0.58	28.67±0.33	17.33±0.33	16.67±0.33	28.00±0.58	21.28
	100	22.33±0.33	19.00±0.58	33.00±0.58	19.00±0.58	17.67±0.33	24.00±0.58	22.50
	150	22.67±0.33	21.00±0.58	34.67±0.33	20.76±0.33	19.00±0.58	29.00±0.58	24.52
	200	23.67±0.33	24.00±0.58	36.00±0.58	22.00±0.58	23.00±0.58	33.33±0.33	27.00
Stem	50	19.33±0.33	18.00±0.58	27.00±0.58	16.67±0.33	18.00±0.58	18.67±0.33	19.56
	100	20.33±0.33	19.33±0.33	28.00±0.58	17.67±0.33	19.67±0.33	19.00±0.58	20.67
	150	21.33±0.33	20.00±0.58	30.33±0.33	20.00±0.58	20.33±0.33	28.00±0.58	23.33
	200	22.76±0.33	24.33±0.33	32.33±0.33	22.00±0.58	21.00±0.58	32.00±0.58	25.74
Means Type of Part used		21.85	20.69	30.85	18.85	18.92	24.69	LSD =2.29

Between Strains LSD=0.5774

Table 2.A. RAPD analysis of Gram positive bacteria using A7 random primer

<i>B.subtilis</i> (ATCC11774)			
Band No	Band size (Kb)	Control	Treatment
1	5772.895	-	+
2	4363.625	-	+
3	2015.905	+	+
4	1664.134	+	-
5	1017.085	+	+
6	839.606	+	-
7	756.935	-	+
8	605.72	-	+
9	513.15	+	-
10	410.636	+	-
11	376.004	-	+
12	256.23	-	+
13	176.428	-	+
Total=13	13	6	9
<i>S.aurues</i> (ATCC29213)			
Band No	Band size Kb)	Control	Treatment
1	3139.924	+	+
2	2521.896	+	-
3	1820.121	+	+
4	879.714	+	+
5	509.944	+	+
6	411.768	-	+
7	306.871	-	+
8	214.486	+	+
Total=8	8	6	7
<i>M.luteus</i> (ATCC4698)			
Band No	Band size (Kb)	A7 5	
		Contr ol	Treatment
1	2528.518	-	+
2	2060.894	-	+
3	1418.981	+	-
4	859.787	-	+
5	531.724	+	-
6	322.182	+	+
7	162.4	+	+
Total=7	7	4	5

RAPD analysis of Gram -ve bacteria using A7 random primer.

Primer A7 with *P.aeruginosa* ATCC27853 revealed 12 fragments, where 7 fragments were polymorphic with size ranged from 1362.547 to 227.207 Kb and the other residual 5 fragments were commonly detected in the two samples (Fig 2 and Table 2.B). The total band number of the treated sample was lower than the control (5 Vs .12). However, 7 fragments were uniquely displayed in the control sample and disappeared in the treatment. Therefore, both two types of fragments clearly showed the effects of the treatment. Primer A7 with *K.pneumonia* (ATCC700603) revealed 11 fragments whereas 6 fragments were polymorphic with size ranged from 4840.914 to 289.958 Kb and 5 fragments were common (Fig 2 and Table 2.B). The total band number of the treated sample was closely equal to the control (7 Vs .9). It is interesting to observe that 2 fragments were uniquely displayed in the treatment *K.pneumonia* with 100 µl Ethanolic extract of *Conocarpus erectus*, while other 4 fragments were detected in control and disappeared in the treated sample .Both two types of fragments clearly showed the effect of the treatment. Primer A7 with *E.coli* (ATCC8739) revealed 11 fragments whereas all fragments were polymorphic with size ranged from 4310.891 to 168.373 Kb (Fig Table 2). The total band number of the treated sample was higher than the control (8 Vs .3). However, 8 fragments were uniquely displayed in the treated sample, while other 3 fragments were detected in control and disappeared in the treated sample. Where the 8 induced fragments were induced under treatment and the three other fragments were disappeared and replaced by fragment number eight.

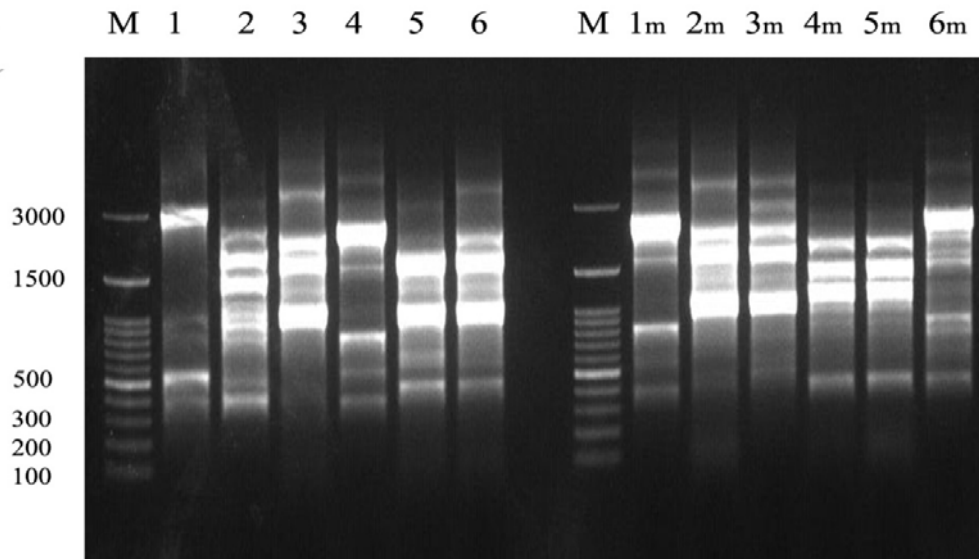


Figure 2. RAPD patterns for 6 strains of Bacteria obtained with B5 random primers :1,control *B.s* ATCC 11774;2, control *S.aureus* ATCC 29213;3,control *M.luteus* ATCC 4698 ; 4, , control *P.aeruginosa* ATCC 27853;5,*K.Pneumonia* ATCC700603 ;6,*E.coli* ATCC 8739;1m,Treated *B.s*;2m,Treated *S.aureus*;3m,Treated *M.luteus*;4m, Treated *P.aeruginosa*;5m,Treated *K.Pneumonia*;6m, *E.coli*

Table 2.B. RAPD analysis of Gram negative bacteria using A7 random primer

<i>Paeruginosa</i> (ATCC27853)			
Band No	Band size Kb)(Control	Treatment
1	1362.547	+	+
2	941.74	+	+
3	2462.996	+	+
4	913.578	+	-
5	1262.957	+	-
6	1577.902	+	-
7	2317.892	+	-
8	3077.198	+	-
9	4659.654	+	-
10	631.43	+	-
11	550.797	+	+
12	227.207	+	+
Total12	12	12	5
<i>K.penumonia</i> (ATCC700603)			
Band No	Band size Kb)(Control	Treatment
1	4840.914	+	+
2	8835.777	+	-
3	6470.229	+	-
4	2420.727	+	+
5	1921.403	+	+
6	1172.101	+	-
7	822.194	+	+
8	480.458	-	+
9	381.354	+	-
10	171.271	+	+
11	289.958	-	+
Total=11	11	9	7

Band No	Band size (Kb)	<i>E.coli</i> (ATCC8739)	
		Control	Treatment
1	4310.821	-	+
2	2831.985	-	+
3	1811.028	-	+
4	1303.842	+	-
5	1034.268	-	+
6	713.212	-	+
7	565.753	+	-
8	339.147	-	+
9	248.146	-	+
10	222.802	+	-
11	168.373	-	+
Total=11	11	3	8

RAPD analysis of Gram +ve bacteria using B5 random primer

Primer B5 with *B.subtilis* (ATCC11774) revealed 10 fragments, whereas 9 fragments were polymorphic with size ranged from 6012.776 to 283.505 Kb and 1 fragments were common (Fig 2 and Table 3.A). The total band number of the treated sample was closely equal to the control (6 Vs .5). However, 5 fragments were uniquely displayed in the treatment sample and displayed in the treatment, while other 4 fragments were detected in the control and disappeared in the treated sample. Therefore both of the two fragments types clearly showed the effect of the treatment. Primer B5 with *S.aureus* (ATCC29213) revealed 6 fragments, whereas 15 fragments were polymorphic with size ranged from 5554.323 to 489.844 Kb and 4 fragments were common (Fig2 Table 3.A). The total band number of the treated sample was closely near to the control (6 vs.4). However, 2 fragments were uniquely displayed in the treatment sample, while other 4 fragments were detected in the control and treatment sample. Therefore both treatments where induced fragments under treatment.

Primer B5 with *M.luteus* (ATCC4698) revealed 13 fragments were polymorphic with size ranged from 4906.114 to 373.418 Kb (Fig2 Table 3.A). The total band number of the treated sample was closely near to the control (6 vs.7). However, 6 fragments were uniquely displayed in the treatment sample, while other 7 fragments were detected in the control and disappeared in treated sample. Therefore, both of the fragments clearly showed the effect of the treatment, where the six induced fragments were induced under treatment and the seven other fragments were disappeared and replaced by the other six.

Table 3.A. RAPD analysis of Gram + ve bacteria using B5 random primer

Band No	Band size (Kb)	<i>B.subtilis</i> (ATCC 11774)	
		Control	Treatment
1	6012.776	-	+
2	3240.037	+	+
3	1868.799	-	+
4	895.402	+	-
5	762.432	-	+
6	669.593	+	-
7	491.528	-	+
8	379.112	+	-
9	360.816	-	+
10	283.505	+	-
Total=10	10	5	6
Band No	Band size (Kb)	<i>S.aureus</i> (ATCC29213)	
		Control	Treatment
1	5554.323	-	+
2	4347.167	+	+
3	2463.169	+	+
4	1823.4	+	+
5	1033.165	+	+
6	489.844	-	+
Total=6	6	4	6

Band No	Band size (Kb)	<i>M.luteus</i> (ATCC4698)	
		Control	Treatment
1	4906.114	-	+
2	3842.811	+	-
3	3400.985	-	+
4	2621.787	+	-
5	2433.938	-	+
6	1947.359	+	-
7	1751.139	-	+
8	1371.614	+	-
9	1342.786	-	+
10	997.368	+	-
11	589.569	+	-
12	437.908	-	+
13	373.418	+	-
Total =13	13	7	6

Table 3.B. RAPD analysis of Gram - ve bacteria using B5 random primer

Band No	Band size (Kb)	<i>P.aeruginosa</i> (ATCC27853)	
		Control	Treatment
1	2584.406	+	+
2	1851.405	+	+
3	1553.777	+	-
4	1493.489	-	+
5	1274.839	+	-
6	1151.492	-	+
7	1069.899	+	-
8	934.15	+	-
9	824.9	+	-
10	740.887	+	-
11	423.333	+	-
12	330.105	+	-
Total	12	10	4
Band No	Band size (Kb)	<i>K.pneumonia</i> (ATCC29213)	
		Control	Treatment
1	7605.217	+	-
2	5163.37	-	+
3	4809.945	+	-
4	3319.464	-	+
5	2639.868	+	-
6	2379.998	-	+
7	1821.839	+	-
8	1763.184	-	+
9	1327.776	-	+
10	983.662	+	-
11	916.332	-	+
12	716.904	+	-
13	484.076	+	-
14	429.341	-	+
15	328.651	+	-
Total	15	8	7

Band No	Band size Kb	<i>E. coli</i> (ATCC4698)	
		Control	Treatment
1	6715.413	-	+
2	5055.914	+	-
3	4538.955	-	+
4	3417.297	-	+
5	2817.456	+	-
6	2723.104	-	+
7	2157.647	+	+
8	1904.321	-	+
9	1433.729	+	-
10	1025.664	+	-
11	991.316	-	+
12	826.642	-	+
13	452.875	-	+
14	413.553	+	-
Total=14	14	6	9

RAPD analysis of Gram -ve bacteria using B5 random primer

Primer B5 with *Paeroginosa* (ATCC27853) revealed 12 fragments, whereas 8 fragments were polymorphic with size ranged from 2584.406 to 330.105 Kb and 2 fragments were common (Fig 2 and Table 3.B). The total band number of the treated sample was lower than the control (4vs .10). It is interesting to note that 2 fragments were uniquely displayed in the treatment *Paeroginosa* (ATCC27853) with 100 µl ethanol extract of *Conocarpus erectus*, while other 8 fragments were detected in the control and disappeared in the treated sample. Both two types of fragments clearly showed the effect of the treatment.

Primer B5 with *K.pneumonia* (ATCC700603) revealed 15 fragments were polymorphic with size ranged from 7605.217 to 328.651 Kb (Fig and Table). The total band number of the treated sample was closely near to the control (7 vs .8). However, 7 fragments were uniquely displayed in the treatment sample with 100 µl ethanolic extract of *Conocarpus erectus*, while other 8 fragments were detected in the control and disappeared in the treated sample. Where the seven induced under treatment and the eight other fragments were disappeared and replaced by the seven one.

Primer B5 with *E.coli* (ATCC700603) revealed 14 fragments, whereas 13 fragments were polymorphic with size ranged from 6715.413 to 413.553 Kb and 1 fragments were common (Fig 2 and Table 3.B) . The total band number of the treated sample was higher than the control (9 vs .6). However, 8 fragments were uniquely displayed in the treatment sample, while other 5 fragments were detected in the treated samples, both two types of fragments clearly showed the effect of the treatment. Worth mentioning that as far as we know this present study is the first to report on the RAPD method utilization on Bacteria strains treated with plant species parts ethanol extracts, definitely it is the case for those of *Conocarpus erectus* with the bacteria strains studied presently.

In the present study RAPD assays detected DNA damage, caused by the plant extracts at different doses. However, the interpretation of the molecular events responsible for differences in the RAPD patterns is not an easy task since different DNA alterations can induce similar type of changes. (Lalroluanga *et al.*, 2011). Exposure of an organism to these plant extracts may result in the formation of covalently bound adducts between the chemical or its metabolites and the DNA; faulty repair of these adducts often results in mutations and, sometimes, cytogenetic changes. In this contest, present results showed that the fragments were displayed in the control and disappeared in the treated bacteria strains. Therefore it can be stated that RAPD could be of great help in the explanation of the antibacterial mode of action of ethanol extract of *Conocarpus erectus* on DNA synthesis. The effects seen in the present study may reflect a chemical and physiological changes in tested bacteria strains, this change of gene expression clearly showed the effect of change in nucleic acid, and may also reveals anew genotypes (Miki *et al.*, 2001). Therefore more rigors investigation is needed in this matter.

Disappearing bands are likely to be due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Nelson *et al.*, 1996; Liu *et al.*, 2005; Enan, 2006; Liu *et al.*, 2009). Total or partial changes in DNA sequence, due to mutation and/or large deletions create in new priming sites can be induced by effect-induced genotoxicity eg, see Enan (2006) and Maryam *et al.* (2010). Our finding support this claim that DNA polymorphisms detected by RAPD can be considered as a powerful biomarker assay for detection of the level of DNA damage in various treated bacterial strains to ethanol extracts of *Conocarpus erectus* at different concentrations.

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