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Journal of Chemical and Pharmaceutical Research, 2015, 7(3):152-157

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Molecular characterization of *Cissus quadrangularis* by RAPD technique

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ABSTRACT

Cissus quadrangularis is an ancient medicinal plant native to the hotter parts of Srilanka and India. Cissus quadrangularis has been used by common folk in India for promoting the fracture healing process. Cissus quadrangularis belongs to the family Vitaceae and genus Cissus Linn, used for the treatment of antihelmintic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation, asthma. The present paper deals with the molecular assay of Cissus quadrangularis L. The present investigation deals with before and after inoculation of pathogen E-coli into Cissus quadrangularis at different concentration of inoculums (0.5 ml, 1 ml and 1.5ml). After 24, 48 and 72 hours incubation the plant parts are cut, dried and powdered. The powdered samples taken for molecular assay. The molecular characterization of DNA over the period of incubation shows that the number of bands increases with time in each of the species and are more when compared to control readings. The frequency profile of RAPD bands were studied with respect to UPGMA band analysis using alpha imager software. Comparative study of the molecular assay of DNA profile of the control and the treated one was done to locate the specific site of DNA that could be targeted for Drug designing in future.

Keywords: *Cissus quadrangularis*, *E-coli*, RAPD, Molecular Characterization, UPGMA-Frequency analysis.

INTRODUCTION

Traditional medicine in many areas of the world relies on the use of a wide variety of plant species. In Africa, phytotherapy still plays an important role in the management of diseases, mainly among populations with very low income [1]. *Cissus quadrangularis* Linn (Vitaceae) (C. quadrangularis) originated from India and Malaysia grows in Savannah areas in Africa (Cameroon, Mali, Mauritania, Senegal, Somalia and Chad) [2]. In traditional medicine, the plant is used to treat hemorrhoids, anorexia, indigestion, and asthma, [3]. In Sahelian areas particularly, C. quadrangularis is used in the treatment of sickle cells, syphilis, gonorrhea, fractures, colds, pains, malaria, abscess, asthma and as an analgesic [4]. The plant is also used in Cameroon to treat epilepsy (personal communications). Chemical studies showed the presence of sterols, steroids, tannins, flavonoids, carotenes, ascorbic acid, linoleic acid in C. quadrangularis [5]. Pharmacological studies of fresh leaves and roots showed that C. quadrangularis possesses antioxidants, antibacterial, analgesic and neurosedative activities [6].

EXPERIMENTAL SECTION

Ten *Cissus quadrangularis* plants were selected for the experiment. One plant was kept as control and rest of the plants were incubated by injecting 2 ml culture each with human pathogenic microorganisms (*P. aeruginosa)* at different time intervals. The organisms were inoculated in proper culture for a specific period of time. After

appropriate incubation time leaves were collected and DNA was isolated by two different methods i.e cTAB method and phenol: isoamylalcohol method. The isolated DNA was run on gel and its purity and DNA content was checked via Nanodrop technique. Molecular characterization was done of the obtained samples via RAPD and verified by running the gel, developing matrix chart and dendogram.

2.1 ISOLATION OF DNA FROM PLANT SAMPLE:

Procedure:-

2g of sample + 5 ml of homogenizing buffer (grind 5 minutes). Add 15ml of lysis buffer (grind 5 minutes). Incubate at 65^0 C for 20 minutes. Centrifuge at 8000 rpm for 10 minutes. Take 500 ul of supernatant + equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1). Centrifuge at 12000 rpm for 10 minutes. Take supernatant + double volume of child ethanol. Centrifuge at 12000 rpm for 10 minutes. Collect pellet + 25 ul TE.

2.2 GEL ELECTROPHORESIS:-

Gel used:-

1% Agarose (Isolated DNA); 2% Agarose (RAPD/ PCR)

Running the gel:-

Load the gel and buffer in electrophoretic chamber. Pre run the gel before loading the sample at 50V for 5 minutes. Load the sample into wells. Run at 50V for 10 minutes. Then run at 100V for 30 minutes.

2.3 PCR(RAPD):-

Procedure:-

The sample was prepared using, sterile water; Taq buffer; dNTP; Primers; DNA Template; Taq polymerase. The cycles were adjusted and the sample was run for obtaining PCR products.

PCR:-

Step- 1:- 94^0 C for 5 minutes; **Step- 2:-** 94^0 C for 45 sec, 35^0 C for 1 min, 72^0 C for 1.5 minutes; **Step- 3:-** 94^0 C for 45 sec, 38^0 C for 1 min, 72^0 C for 1 min; **Step- 4:-** 72^0 C for 10 minutes.

RESULTS AND DISCUSSION

The molecular characterization of DNA over the period of incubation shows that the number of bands increases with the incubation period.

E.coli showed less bands with primer 1-2. It showed no result for primer 6 for incubation time at 48 and 72 hours. Similar results were seen for primer 10. For Primer 9 no bands were seen for 72 hours. The possibility for such results may be the degradation of DNA by the pathogen or non specificity of primer to bind with the modified DNA. With increase in incubation period there is no such variation with the characterization. Thus, its effect is almost constant at various incubation periods. Maximum variations in band matrix was seen for primer 4,8 and 9. Maximum similarity was seen for primer 3 and 7.

The amount of DNA is less as compared to control DNA, but as the incubation period increases there is increase in amount of DNA. The probable reason for it may be integration of bacterial DNA with the host DNA or increased level of infection over the period. Increase in 260/280 & 260/230 ratio over the increased period of incubation.

Increase in band number with the incubation period may be due to binding of primers with bacterial DNA along with the host DNA. The exact reason can only be concluded after doing genome sequencing of the plant.

Dendogram and matrix analysis of RAPD samples was done by using alpha imager software using UPGMA-Frequency analysis method. The analysis showed that as there is large similarity among the samples over the incubation period for *E-coli* injected samples. This shows that increasing the incubation period has effect on the samples of *E-coli* shows no profound effect with time.

The samples can be used to check the anti microbial activity of the plant. Also, sequencing of plant genome can be carried out for further analysis.

Figure (1): Control

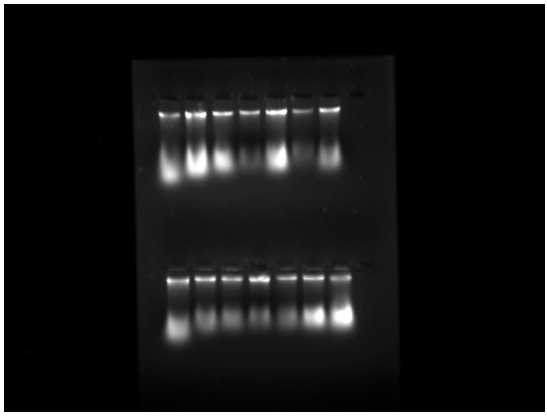
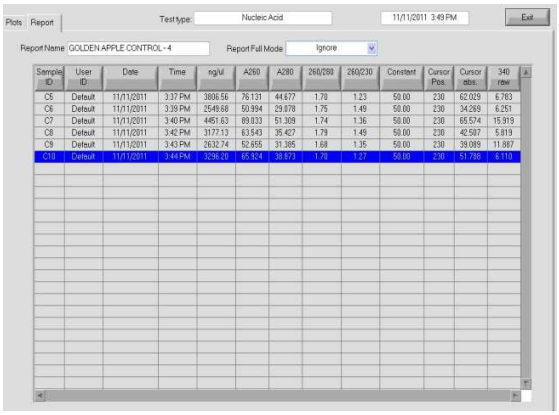
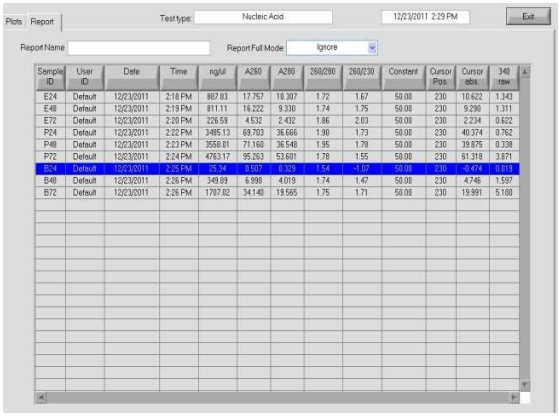


Figure (2): Gel showing isolated DNA of control and injected samples



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs	340 raw
C5	Default	11/11/2011	3:37 PM	3806.56	76.131	44.677	1.70	1.23	50.00	230	62.029	6.783
C6	Default	11/11/2011	3:39 PM	2549.68	50.994	29.878	1.75	1.49	50.00	230	34.269	6.251
C7	Default	11/11/2011	3:40 PM	4451.63	89.033	51.309	1.74	1.36	50.00	230	65.574	15.919
C8	Default	11/11/2011	3:42 PM	3177.13	63.543	35.427	1.79	1.49	50.00	230	42.597	5.819
C9	Default	11/11/2011	3:43 PM	2632.74	52.655	31.385	1.68	1.35	50.00	230	39.089	11.867
C10	Default	11/11/2011	3:44 PM	3296.20	65.924	39.873	1.70	1.27	50.00	230	51.786	6.110

Figure (3): Nanodrop image of control samples



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs	340 raw
E24	Default	12/23/2011	2:18 PM	867.83	17.757	10.307	1.72	1.67	50.00	230	10.622	1.343
E48	Default	12/23/2011	2:19 PM	811.11	16.222	9.330	1.74	1.75	50.00	230	9.290	1.311
E72	Default	12/23/2011	2:20 PM	226.59	4.532	2.432	1.86	2.03	50.00	230	2.234	0.622
P24	Default	12/23/2011	2:22 PM	3485.13	69.703	36.666	1.90	1.73	50.00	230	40.374	0.762
P48	Default	12/23/2011	2:23 PM	3558.01	71.160	36.548	1.95	1.78	50.00	230	39.875	0.338
P72	Default	12/23/2011	2:24 PM	4763.17	95.263	53.601	1.78	1.55	50.00	230	61.318	3.871
B24	Default	12/23/2011	2:25 PM	25.14	0.507	0.329	1.54	-1.07	50.00	230	-0.474	0.819
B48	Default	12/23/2011	2:26 PM	349.69	6.998	4.019	1.74	1.47	50.00	230	4.746	1.597
B72	Default	12/23/2011	2:26 PM	1787.82	34.140	19.565	1.75	1.71	50.00	230	19.991	5.180

Figure (4): Nanodrop image of injected samples

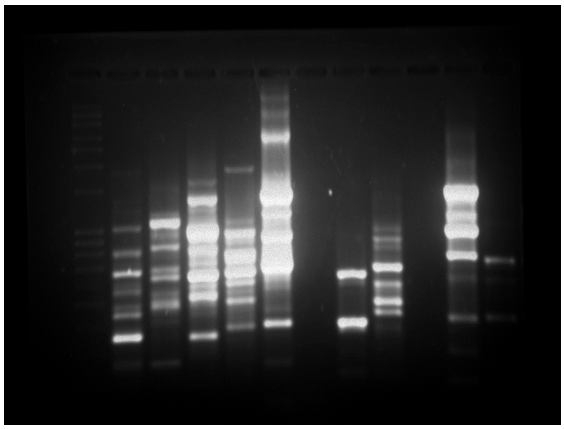


Figure (5): RAPD of control with 10 primers

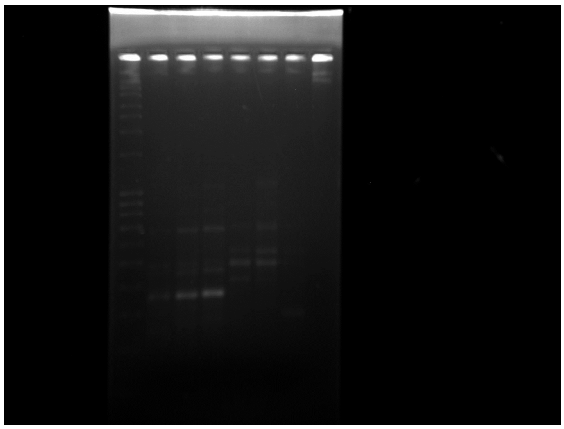
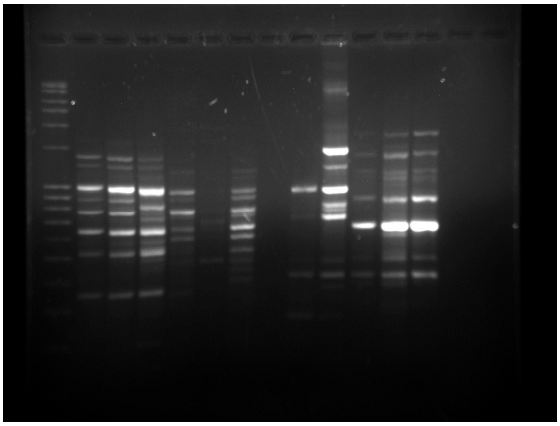


Figure (6): RAPD of plants injected with *E-coli* for 24,48,72 hours with Primer 1,2



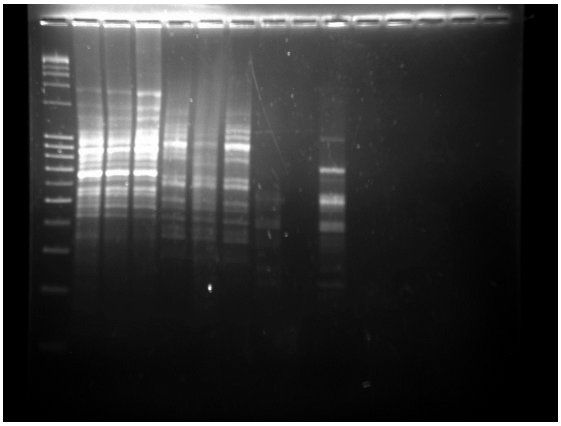


Figure (7): RAPD of plants injected with *E-coli* for 24,48,72 hours with Primer 3,4,5,6
Figure (8): RAPD of plants injected with *E-coli* for 24,48,72 hours with Primer 7,8,9,10

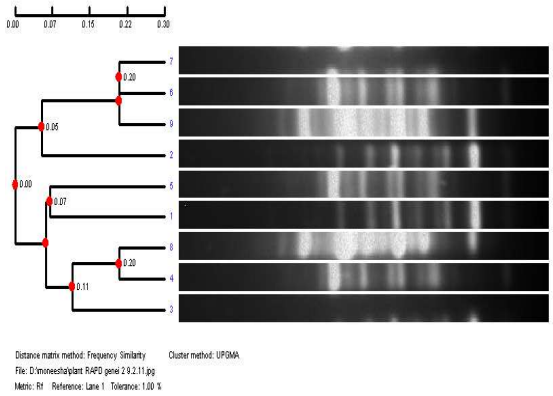


Figure (9): Dendogram for control primers

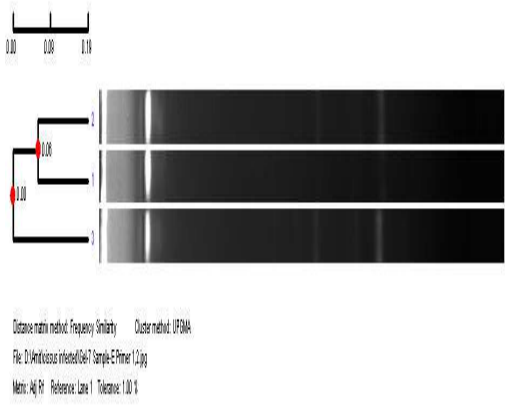


Figure (10): Dendogram for *E-coli* for 24,48,72 for Primer 1

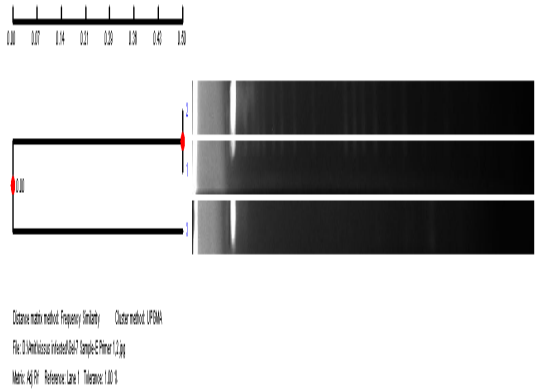


Figure (11): Dendogram for *E-coli* for 24,48,72 for Primer 2

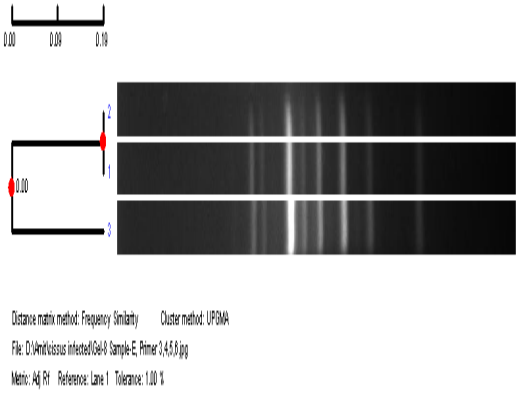


Figure (12): Dendogram for *E-coli* for 24,48,72 for Primer 3

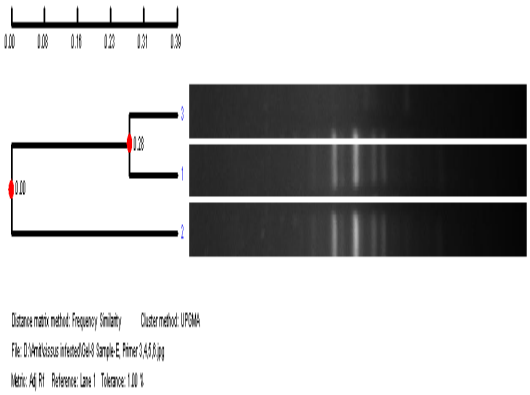


Figure (13): Dendogram for *E-coli* for 24,48,72 for Primer 4

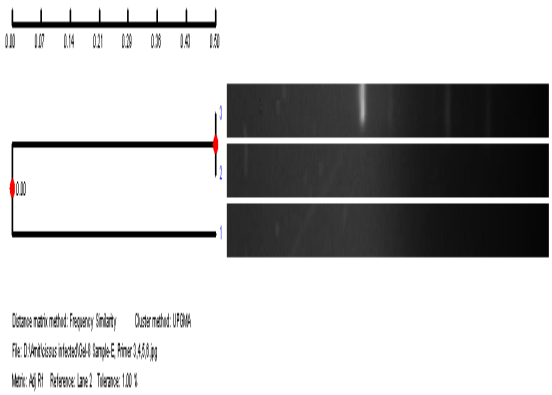


Figure (14): Dendogram for *E-coli* for 24,48,72 for Primer 5

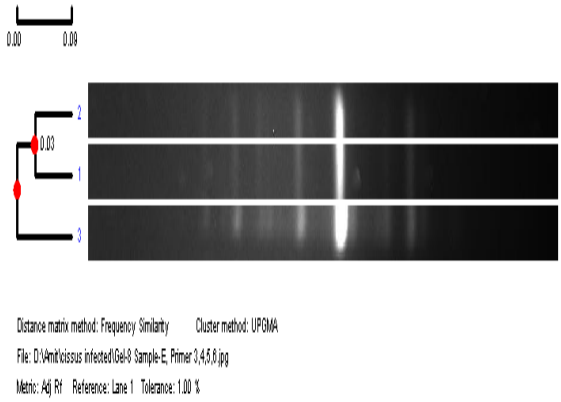


Figure (15): Dendogram for *E-coli* for 24,48,72 for Primer 6

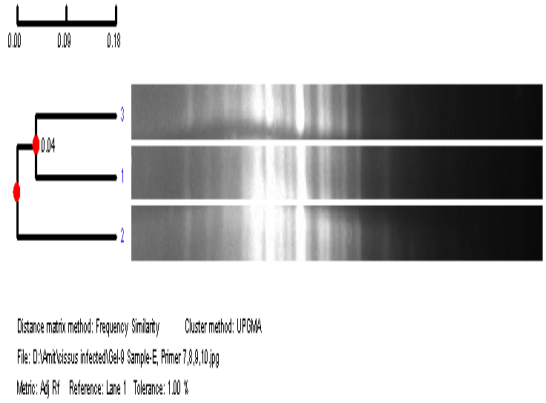


Figure (16): Dendogram for *E-coli* for 24,48,72 for Primer 7

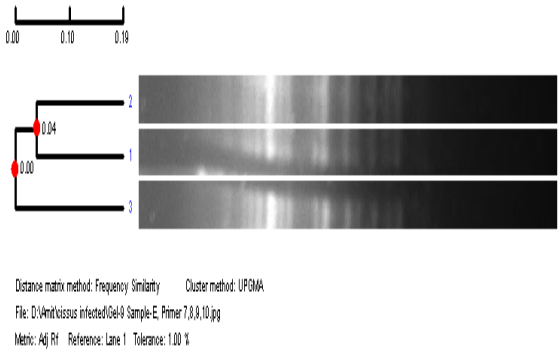


Figure (17): Dendogram for *E-coli* for 24,48,72 for Primer 8

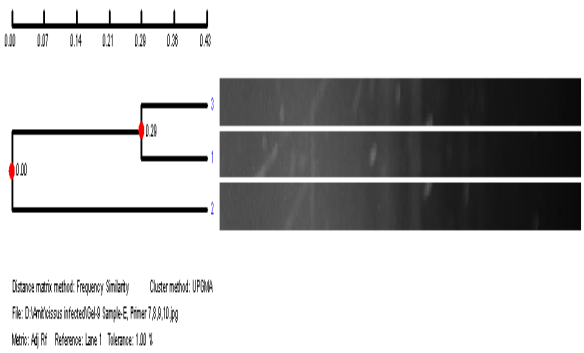


Figure (18): Dendogram for *E-coli* for 24,48,72 for Primer 9

CONCLUSION

The result of the research was evidence of the plants extract having an anti microbial property and hence the work is extended towards production of suitable drugs from the plant parts.

Acknowledgement
I would like to acknowledge Dr. Geetha and director of Sangenomics pvt. Ltd labs. Dr. Prasad for guiding us through the project and providing research facilities in their lab.

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