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°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°C for 5 minutes; Step- 2:- 94°C for 45 sec, 35°C for 1 min, 72°C for 1.5 minutes; Step- 3:- 94°C for 45 sec, 38°C for 1 min, 72°C for 1 min; Step- 4:- 72°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

Figure (3): Nanodrop image of control samples

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
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.

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REFERENCES


