African Journal of Biotechnology Vol. 8 (3), pp. 447-450, 4 February, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effect of *Cassia hirsuta* (L) extract on DNA profile of some microorganisms

M. K. Oladunmoye, F. C. Adetuyi and F. A. Akinyosoye

Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Accepted 11 August, 2006

The effect of ethanol extract of leaf of *Cassia hirsute* (L) on the DNA profile of some selected pathogenic microorganisms were investigated using PCR-RAPD analysis to generate DNA fingerprint. The change in molecular configuration of organisms with and without extract shows a wide disparity between the sensitive and the resistant organism. Thus, this study demonstrates that some of the organisms are susceptible to the antimicrobial activity of *C. hirsute* extract while others are resistant.

Key words: *Cassia hirsuta*, DNA finger printing, antimicrobial activity.

INTRODUCTION

Phytomedicine is the use of (medicinal) plant parts or extracts, wholly or partly to heal illnesses especially in man. The act which is as old as creation started by trial an error when the early man discovered that certain plants have some efficacy in healing (Ali et al., 1999). Today, the advancement in science has enabled us to identify the chemical constituents within these plants and so can better understand their heading powers.

Plants as gifts of nature have many therapeutic properties combined with much nutritive value, which have made their use in chemotheraphy as valuable as the synthetic drugs. People have always relied on plants for food to nourish and sustain the body. Herbal organs or systems of the body are used to 'feed' and restore to health those parts, which have become weakened. *Cassia hirsute* (L) is a medicinal plant widely used for stomach troubles, dysentery, abscesses, rheumatism, haematuria, fever and other diseases. The seed contain a phytotoxin, tannis and 0.25% chrysarobin (Irwin and Barneby, 1982).

EXPERIMENTAL

Plant material

The leaves of *C. hirsute* was collected from Shagari Village, Akure, Ondo State, Nigeria in July 2001 and identified by Mr S. A. Aduloju. Voucher specimen was deposited at the herbarium of Department of forestry and wild life of the Federal University of Technology, Akure, Ondo State, Nigeria.

Preparation of extract

The air-dried leaves were grinded into fine powder and extracted with 60% ethanol. The filtrate concentrated *in vacuo* using rotary evaporator.

Microorganisms

Eight-typed bacteria were obtained from Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The bacteria are *Proteus vulgaris* (NCIB 67), *Bacillus subtilis* (NCIB 3610), *Clostridium sporogenes* (NCIB 5), *Shigella dysentriae* (NCIB 8867), *Klebsiella pneumoniae, Serratia marcensce, Pseudomonas aeruginosa* (NCIB 450) *and Escherichoa coli* (JM109). The organisms were inoculated into nutrient broth and incubated at 37°C for 18 h before adjustment with McFarland turbidity standard as described by Thrupp (1980) to adjust to 10^6 cfu/ml.

DNA preparation

A crucial step in the RAPD procedure is the preparation of pure unfragmented, essentially plamid-free DNA. The following procedure described by Monstein et al. (1996) with a little modification was used in this study. Cells grown on broth were pelleted by centrifugation, washed with 1 ml TE buffer (10) mM Tris, 10 mM EDTA, pH 8.0), centrifigued and resuspend in 325 µl of lysis buffer (TE buffer containing 2 mg/ml lysozyme and 8.5 U/ml of

*Corresponding author E-mail: chourlar@yahoo.com.

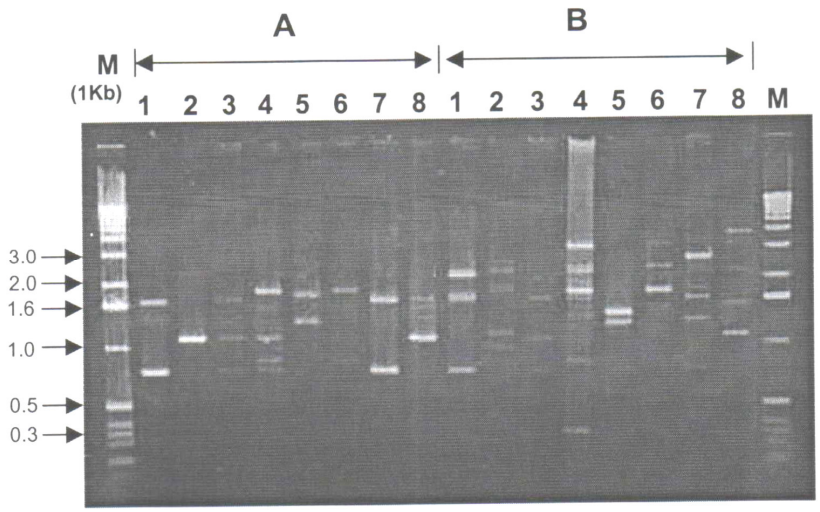

Figure 1. PCR DNA fingerprinting. A = Extract of the organisms; and B = organisms only (control). 1 = *Proteus vulgaris*, 2 = *Bacillus subtilis*, 3 = *Clostridium sporogenes*, 4 = *Shigella dysentriae*, 5 = *Serratia marcensce,* 6 = *Klebsiella pneumoniae,* 7 = *Pseudomonas aeruginosa* and 8 = *Escherichia coli*.

mutanolysin). The tube was mixed, 30 µl of 20% SDS (sodium deodycyl sulphate) and 3 µl proteinase K (20 mg/ml) was added, the tube was mixed and incubated at 37°C for 1 h. The DNA was extracted with 1 volume phenol : chloroform : isoamyl-alcohol (25:24:1), and precipitated with 1 volume ice-cold ethanol. The tube was then incubated at -70°C for 15 min. The DNA was pelleted by centrifugation, washed twice with 70% ethanol and once with absolute ethanol, and air-dried. The pellet was redissolved in 50 µl TE TNAse buffer (10 nM EDTA, pH 7.5, 0.5 mg/ml RNAse).

PCR amplification of random DNA fragment

RAPD-PCR was carried out in 25 µl reaction mix using 1 µl of purified DNA, Boehringer-Mannheim Standard PCR buffer (pH 8.3, 1.5 mM $MgCl_2$), 0.2 mM of each nucleotide (Perkin Elmer, Branchburg, NJ, USA) and 2.5 Units of Taq polymerase (Boehringer - Mannheim, Germany). The primers were tried at two different concentration; 4 µM and 8 µM. Six different primers were tested: 5'-ACGGGACCTG-3', 5'-GTCCCGAGGA-3', 5'-TTCCCCGCGA-3', 5'-TTCCGCCACC-3', 5'-AGGCCCATG-3' and 5'-TCGCACCCT-3'. They were synthesized by Operon (Westburg BV) and Roth (Carl Roth GmbH & Co.). The reaction mix was overlaid with mineral oil and cycled through the following temperature profile: 94°C for 2 min followed by 45 cycles of 95°C for 60 s, 36°C for 60 s, 72°C for 2 min. The PCR reaction was terminated at 72°C for 10 min and thereafter cooled to 4°C (Berg et al., 1994).

Gel electrophoresis

Gel Electrophoresis was carried out by applying 10ul of sample to submerge horizontal 1.5% Agarose (TYPE III:EEO, Sigma, St Louis, USA) slab gels. Gels were run for 2.5 h at 100 V in TB electrophoresis buffer (89 mM boric acid, 23 mM H_3PO_4, 2.5 mM EDTA, pH 8.3) without cooling. These running conditions gave sharp, well-separated bands. A DNA molecular weight marker VI (0.5 µg) (Boehvirger-Mannheim Secaandinavia, Bromma, Sweden) was used as standard. After eletrophoresis, the gel were stained in ethidium bromiode (3) µg/ml) for 5 min and thereafter washed for 10 min and visualized at 302 nm with a Ultra-Violet transilluminator (UVP inc., San Gabriel, CA, USA) and photographed.

Reading of patterns, numerical analysis

The patterns were scored by denoting the presence of band (fragment) with; T; and absence of band with 'o'. The data were collected and analysed using Pearson product moment correlation coefficient ® and the unweighted pair group method using arithmetic averages (UPGMA; Romersburg 1984).

RESULTS AND DISCUSSION

RAPD analysis

Six different primers were evaluated, but only one, a 10-mer oligonucleotides primer with 80% G+G contents ((5'-AGGCCGATG-3') gave a sufficient number of bands to perform a proper comparison. The finding that primer with a relatively high G+G content appear to give the best (a satisfactory number of bands for all isolated tested) for microorganisms is in accordance with Berg et al (1994) who found that primers with a higher G+C gave better results in RAPD analysis of *Helicobcter pylori* isolates.

DNA finger printing

Typical band pattern obtained by using RAPD on extracted DNA under the most favourable condition described above are shown in Figure 1. Several organisms' specific bands could be observed and the

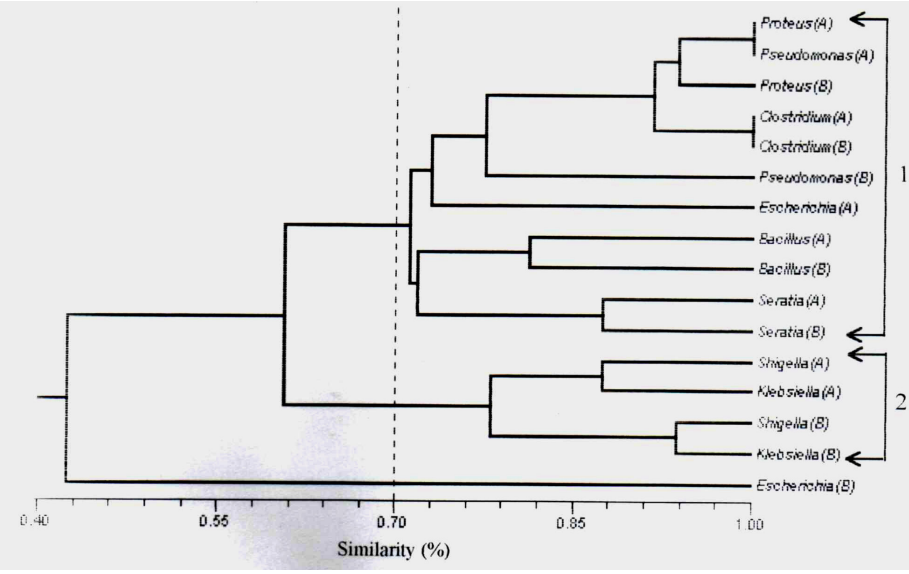

Figure 2. Dendrogram obtained by using RAPD on the organisms treated with *Cassia hirsute* L. extract.

bands of fragments of high molecular weights are found on 4.0 kb while the low molecular weights are on 0.3 kb.

Cluster analysis

The clusters analysis in which the Pearson Product moment correlation coefficient followed by UPGMA were used resulted in the dendrogram shown in Figure 2. The similarity between the organisms treated with the extracts (A) and the control (B) was evaluated based on these RAPD profiles.

The result clearly showed that the extracts of *C. hirsute* has antimicrobial activity on the test organisms as shown by the difference in the DNA profile of sensitive microorganisms before and after the extract was applied. Some particularly the Gram negative was found to be resistance, as the band pattern of their DNA before and after the introduction of the extract remains essentially the same. The resistance of the organisms might be due to the nature of the cell wall of the Gram-negative organisms that have been shown to be more complex and hence impermeable to the extract. The cell well of Gram negative have been shown to contain in addition to the N-acetyl glucosamine and N-acetylmuramic acid that is present in Gram positive bacteria, lipopolysaccharide and teichoic acid. The nature of the cross linking of cell wall materials in Gram positive bacteria is less compact than what is obtainable in Gram-negative. The resistance of some of the organisms may also be as a result of their genetic compositions influenced by the environmental factors. Conjugation has been known since the beginning of antimicrobial resistance studies. It has show recently that transfer of antimicrobial resistance genes in natural environmental can occur between phylogenetically distant bacteria genera (Courvalin, 1994).

The marked effect of the extract on *E. coli* and *P. aeruginosa* though a Gram negative bacteria might be that they are unable to build up any genetic resistance. It has been found that high level resistance is common in *P. aeruginosa* and may be due to mutations which altered the lipoplysaccharide or reduced uptake of the extract (Montie and Patamasucon, 1995).

Conclusion

The extract of *C. hirsute* was found to posses antimicrobial activity against some pathogenic bacteria. The partially purified extract can further be purified for possible clinical and pharmacological trials. The resistance and susceptibility of the bacterial also depict the spectrum of activity of the extract i.e. it is not a broad spectrum antimicrobial agent. There is also the need to further investigation in tracking down the gene responsible for the resistance and susceptibility using molecular markers with specific primer rather than random primers.

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