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Effect of *Cassia hirsuta* (L.) Extract on DNA Profile of Some Microorganisms

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Abstract: The effect of ethanolic leaf extract from *Cassia hirsuta* (L.) on the DNA profile of some selected pathogenic microorganisms were investigated using PCR-RAPD analysis to generate DNA fingerprints. The change in molecular configuration of organisms with and without extract shows a wide disparity between the sensitive and the resistant organisms. Thus, this study demonstrates that some of the organisms are susceptible to the antimicrobial activity of *Cassia hirsuta* extract while some are resistant. The mechanism of action of the extract or other antimicrobial agent on these organisms is due to alteration on the genome and the extract is mutagenic on the susceptible organisms.

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

Introduction

In recent years, acquired resistance to most commonly available standard antibiotics (like chloramphenicol and ampicillin) by most commonly encountered pathogenic microbes like *Shigella dysenteriae* and *Haemophilus influennzae* type b is a growing world wide problem (Monica, 2000). It has gotten to the extent that one could correctly predict the resistance of certain organisms to specific antimicrobials. Edeoga *et al*. (2005) has stated that Proteus species are generally resistant to nitrofurantoin and tetracycline, *Streptococcus pyogenes* to penicillin, *Klebsiella pneumoniae* to ampicillin and anaerobes to metronidazole. There have also been reported cases of drug hypersensitivity and many third generation antibiotics have prolific side effects on patients' normal flora. Protracted use of these refined drugs also results in immunosuppression. (Prescott *et al*., 2005).

It has therefore become expedient that an alternative therapeutic measure be adopted. Phytomedicine is the use of (medicinal) plant parts or extracts, wholly or partly to heal illnesses in animals especially 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 of science has enabled us to identify the chemical constituents within these plants and so can better understand their healing powers.

Plants as gifts of nature have many therapeutic properties combined with much nutritive value, which have made their use in chemotherapy 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 hirsuta* (L.) is a medicinal plant widely used for stomach troubles, dysentery, abscesses, rheumatism, haematuria, fever and other diseases. The seed contain phytotoxin oil, tannin and 0.25% chrysarobin (Irwin and Barneby, 1982).

However, there has not been any information on the possible effect of the extract on the DNA profile of some microorganisms in order to establish the likely mechanism of action. This knowledge will also give an insight into susceptibility or resistivity of these organisms to other antimicrobial agents used in chemotherapy. The present research is therefore designed to examine the DNA pattern of some

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selected pathogenic microorganisms subjected to antimicrobial activity of the ethanolic leaf extract from *Cassia hirsuta* in order to access the mode of activity and possible mutagenic potential.

Materials and Methods

Plant Material

The leaves of *Cassia hirsuta* 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 and the filtrate concentrated *in vacuo* using rotary evaporator.

Micro Organisms

Eight-typped 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 a population of 10^6 cfu mL^{-1}.

DNA Preparation

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, pH8.0), centrifuged and resuspend in 325 µL of Lysis buffer (TE buffer containing 2 mg mL^{-1} Lysozyme and 8.5 U mL^{-1} of Mutanolysin). The tube was mixed, 30 µL of 20% SDS (Sodium Deodycyl Sulphate) and 3 µL proteinase K (20 mg mL^{-1}) was added and the tube was mixed and incubated at 37°C for 1 h. The DNA was extracted with 1 volume phebnol: 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^{-1} 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, i.e., 4 µ and 8 µ. Six different primers were tested:

- (5'-ACGGGACCTG-3'); • (5'-GTCCCGAGGA-3');
- (5'-TTCCCCGCGA-3'); • (5'-TTCCGCCACC-3');
- (5'-AGGCCCATG-3') and• (5'-TCGCACCCT-3');

Primers I-vi were chosen because they had a G + C content ranging from 30 to 80% and were synthesized by Operon (Westburg BV) and Roth (Carl Roth GmbH and 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 sec, 36°C for 60 sec and 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 10 µL of sample to submerge horizontal 1.5% Vagaries (TYPE III: EEO, Sigma, St Louis, USA) slab gels. Gel were run for 2.5 h at 100 Volts in TB electrophoresis buffer (89 mM Boric acid, 23mM 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^{-1}) 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

RAPD Analysis

Six different primers were evaluated, but only one, a 10-mere 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 Fig. 2. Several organisms' specific bands could be observed and the bands of fragments of high molecular weights are found on 4.0 kb while the low molecular weights are on 0.3 kb.

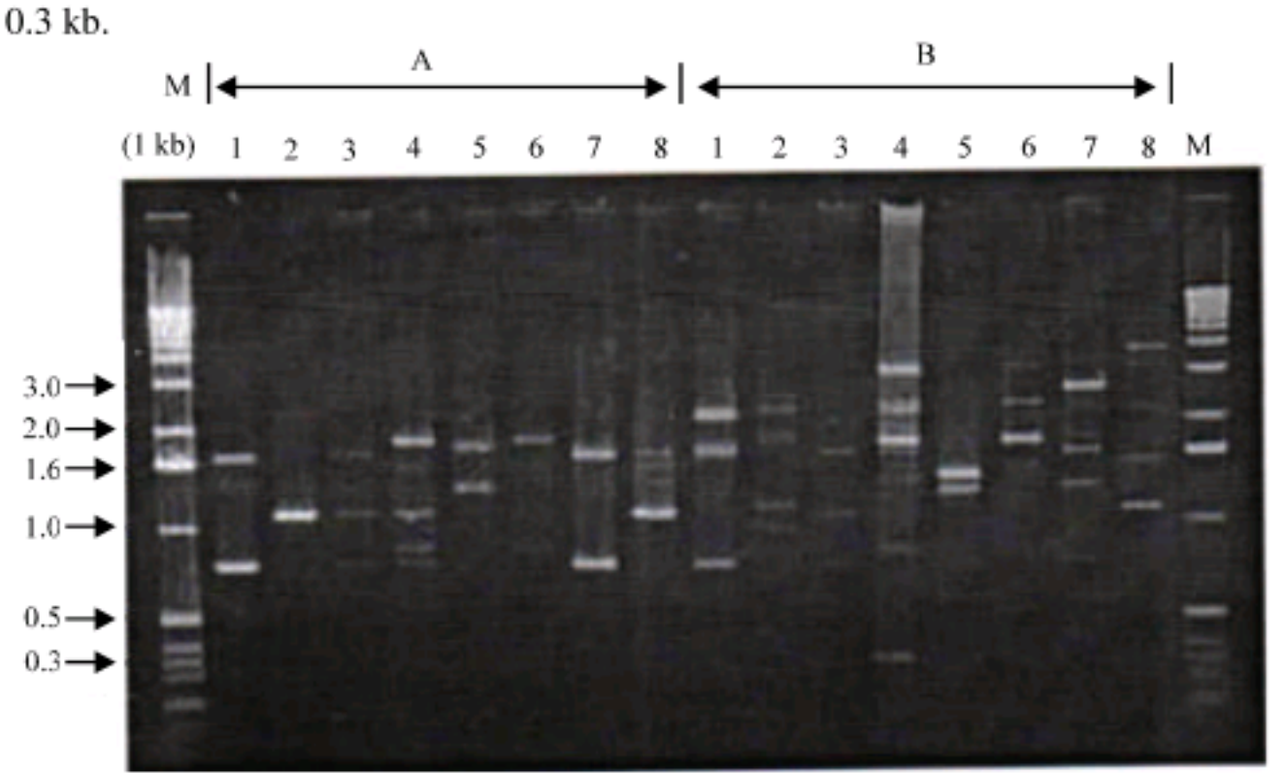

Fig. 1: PCR DNA fingerprinting A = Extract and the orginisims, B = Organism only (control), 1 = *Proteus vulgeris*, 2 = *Bacillus subtilis*, 3 = *Clostridium sporogenes*, 4 = *Shigella dysenteriae,* 5 = *Sewatia marcescens*, 6 = *Klebsiella pneumonia*, 7 = *Pneumonas aeruginosa*, 8 = *Escherichia coli*

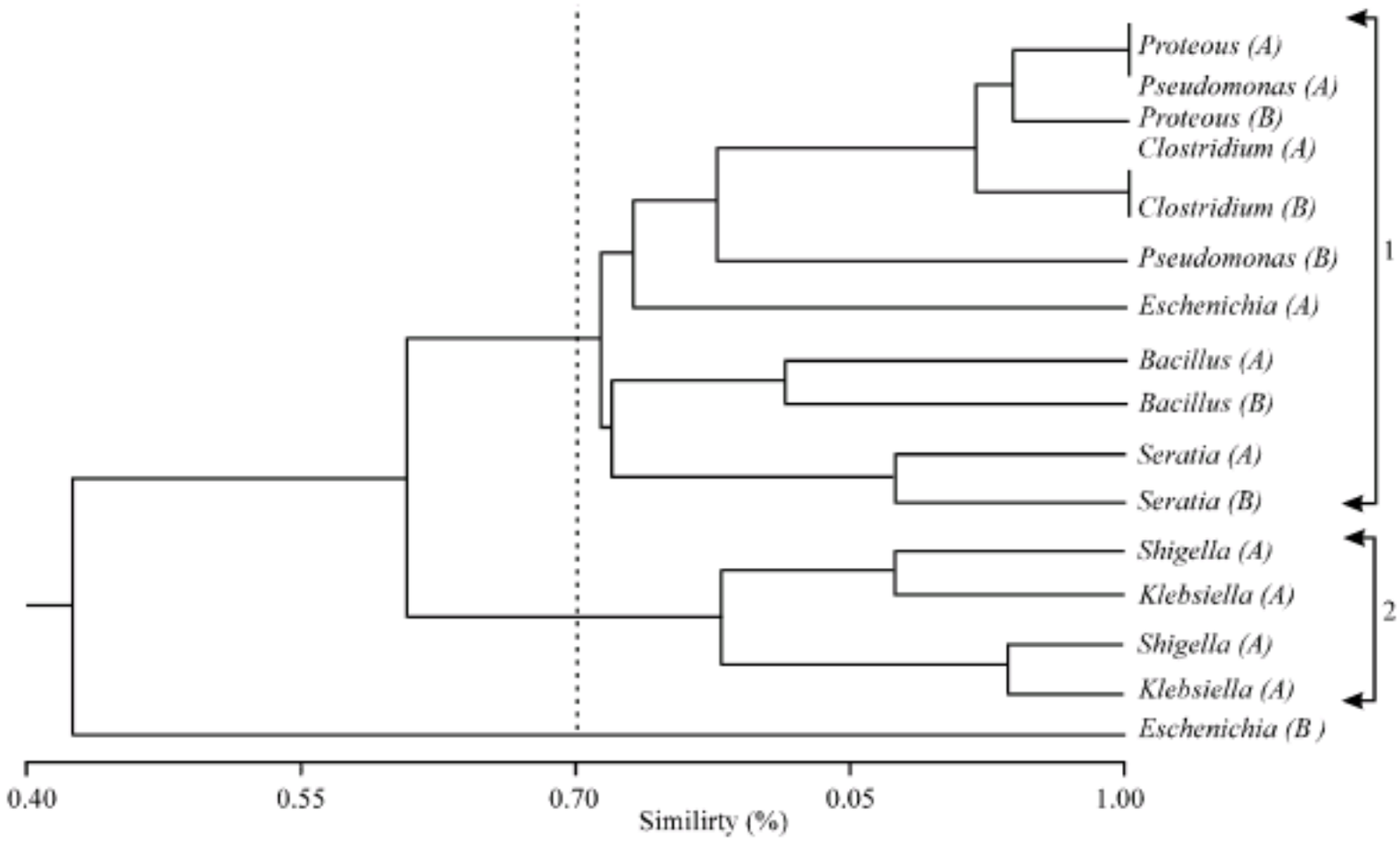

Fig. 2: Dendrogram obtained by using RAPD on the organisms treated with *Cassia hirsuta* L. Extract (A) and control (B)

Cluster Analysis

The clusters analysis in which the Pearson Product moment correlation coefficients followed by UPGMA were used resulted in the dendrogram shown in Fig. 2. The similarity between the organisms treated with the extracts (A) and the control (B) was evaluated based on these RAPD profiles (Fig. 1). Two clusters and one single organism treated with the extracts.

Discussion

The result clearly showed that the extracts of Cassia hirsute has antimicrobial activity and 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 became 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 changes in the band patterns between the sensitive and the resistant organisms is an indication that the mechanism of action of the extract may be due to interaction with the nuclear materials. One of the potent means by which antimicrobial agent exert their effects on organisms had been due largely to owing to interaction with nuclear material (Prescott *et al*., 2005). The variation in the DNA profile

of the sensitive organisms with and those without extract suggest that the extract is mutagenic. This is in agreement with the work of Lei *et al*. (2006) while analysing Y-type gene specific markers reported that interaction of chemical mutagens brings about variation in the DNA profile of hexaploid wheat.

Recommendation and Conclusions

The extract of *Cassia hirsuta* was found to posses' antimicrobial activity against some pathogenic bacteria. The observed alteration on the DNA profile showed that the extract can be mutagenic and that the mode of action can be due to alteration in the genomic constituents of the susceptible organisms. 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 marker with specific primer rather than oligonucleotide primer.

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