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Original Research Article

DNA fragmentation induced by the glycosides and flavonoids from *C.coriaria*

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A B S T R A C T

Genomic DNA fragmentation is the hallmark for cell death, unlike in eukaryotic cell, DNA fragmentation in microorganisms is rarely evaluated. In the present study the DNA fragmentation of microorganisms induced by the flavonoids and glycosides from *Caesalpinia coriaria* (Jacq) Willd was carried out. The DNA fragments were observed using Agarose gel electrophoresis. Degradation of genomic DNA due to the activation of endonucleases is one of the early events of bacterial destruction. This phenomenon was tested for the ability of Glycosides and flavonoids compounds to induce the cleavage in whole cell's DNA fragmentation. Pathogenic bacteria like *E.coli, Staphylococcus aureus* and *Klebsiella pneumoniae* were exposed to glycosides, flavonoids of *Caesalpinia coriaria*, at 10, 20, 30, and 40 µg /ml concentration and incubated for 24 hours. *E.coli, Staphylococcus aureus* and *Klebsiella pneumonia* when treated with the glycoside and flavonoids extracts of *Caesalpinia coriaria* for 24 hours showed a marked DNA fragmentation, and no fragmentation was observed in untreated cells. The results confirmed that the antibacterials (flavonoids and glycosides can interact with DNA of the bacteria may play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases and this may explain the inhibitory action on DNA synthesis. Hence the *C.coriaria* possessed antibacterial potential against these microorganisms.

Introduction

In India, around 17,000 species of higher plants, 7500 are known for their medicinal uses (Shiva, 1996). This proportion of medicinal plants is the highest at any country of the world for the existing flora of that respective country. Ayurveda, the oldest medicinal system in Indian sub-continent has alone reported approximately 2000, medicinal plant species, followed by Siddha age-old written document on herbal therapy has reported on the production of 340 herbal drugs and their indigenous uses (Prajapati *et al*., 2003). In modern pharmacopeia, approximately 25 % of drugs are derived from plants and many others are synthetic analogues built on prototype compounds isolated from plant species (Rao *et al*., 2004). The dependence on medicinal plants

is also due to cultural preferences (Cunningham, 1998).

India in its long history has accumulated a rich body of empirical knowledge in the use of medicinal plants for the treatment of various diseases. Chemical studies of Indian medicinal plants provide a valuable material base for the discovery and development of new drugs of natural origin (Qin and Xu, 1998). In contrast to the synthetic drugs, antimicrobial of plant origin are not associated with many side effects and have enormous therapeutic ability to heal many diseases (Iwu et al., 1999).

Numerous research works have been identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004) still there are evidences that some traditional remedies have already produced that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004). The results of this indicate the need for further research into traditional health systems (Romero et al., 2005).

The study also facilitates pharmacological values leading to synthesis of a more potent drug with reduced toxicity (Ebana *et al*., 1991; Manna and Abalaka, 2000). Antimicrobial activites of many plants have been reported by the researchers. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids etc. that are present in these plants (Sher, 2009).

The mechanism of antimicrobial action of plant secondary metabolites is not fully understood but several studies have been conducted in this direction. Flavonoids may act through inhibiting cytoplasmic membrane function as well as by inhibiting of DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydratase activities (Cushnie and Lamb, 2005; Zhang et al., 2008); the isoflavone genistein was able to change cell morphology (formation of filamentous cells) and inhibited the synthesis of DNA and RNA of *Vibrio harveyi* (Ulanowska et al., 2006).

It has been suggested that terpenes promote membrane disruption; coumarins cause reduction in cell respiration and tannins act on microorganism membranes as well as bind to polysaccharides or enzymes promoting inactivation (Ya et L., 1988; Cowan, 1999).

Materials and Methods

Bacterial strains

Standard strains- *Escherichia coli* MTTC 443, *Methicillin resistant Staphylococcus aureus, Klebsiella pneumoniae(*Clinical Isolate*)* were used for the assay, and they were maintained for further study.

Chemicals and reagents

All the chemicals and reagents for the project used were purchased from Hi-Media, Merck, Qualigens and Loba chemie.

Plant sample collection

The Sample leaves of *Caesalpinia coriaria* were collected form Captain Srinivasa Murti Drug and Ayurveda Research Institute, Chennai and were identified from Dr. Jayaraman. The samples were allowed to dry under the shade completely. The leaves were ground into powder which was used for further extraction.

Extract preparation

About 1 kg of dry sample powder was weighed and macerated with 1000 ml of ethanol solvent and kept overnight in shaker. The extract was collected after filtration using Whatmann No. 1 filter paper and was stored. 1000 ml of solvent was added to the residual mixture and incubated in shaker for 24 hours and the extracts were collected again using Whatmann No.1 filter paper. This procedure was repeated again and the extracts were evaporated below 40 ^{0} C, which was used for further pytochemicals extract preparations.

Extraction of flavonoids (Amal et al., 2009).

The ethanol extract powder was defatted with petroleum ether (40-60^{0}C). The extract was then percolated with methanol until exhaustion at 40^{0}C by rotary evaporator. The condensed was partition using ethylacetate. This ethylacetate extraction contained crude flavonoids (Amal et al., 2009).

Extraction of glycosides (Aya *et al.,* 2011).

The ethanol extract powder were extracted three times with methanol at 25 ^{0}C for 24 hours and then concentrated in vacuum. The extract was washed with n-hexane and then the methanol layer was further concentrated to a gummy mass. The later was suspended with water and extracted with equal volume of ethyl acetate to give glycosides extract of the plant (Aya *et al.*, 2011).

Characterization of phytoconstituents from the leaves of *C.coriaria* (Gaw et al., 2002; Srivastava et al., 2004)

The partial characterization of the different phytoconstituents of *C.coriaria* was done on precoated silica gel TLC plates (Merck, USA). The efficient solvent system used for the flavonoids and glycosides was as follows:

1. Flavonoid Extract: Ethyl acetate: formic acid: acetic acid and water in 10:1.1:1.1:2.7 ratio.
2. Glycoside extract: Chloroform, ethylacetate, methanol and water in 1.5:4:2.2:1 ratio

The developed chromatogram plates were viewed under UV 240nm and 360 nm and day light (Gaw et al., 2002; Srivastava et al., 2004)

DNA fragmentation Sambrook et al., (1989)

Sambrook et al., (1989) method followed for the isolation of DNA from pathogenic bacteria were washed three times with cold (4°C) phosphate buffered saline (PBS) and were formed into pellets by centrifugation at 1,500 rpm for 5 min. They are resuspended in cold TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8) to final concentration 5 x 10^{7} cells per ml. Then one volume of the cells were mixed with 10 volumes of lysis buffer (10mM Tris, 100 mM EDTA, 0.5% SDS,pH 8) for 1 hr at 37°C . Proteinase K was added to the lysed cells in a final concentration 0.1 mg/ml and the incubation was continued for 3 hrs at 56 ^{0}C.

The lysates were cooled to room temperature and DNA was precipitated by adding NaCl to a final concentration 5% together with two volumes of cold(-20 ^{0}C) ethanol. The DNA pellet was washed in cooled 70% ethanol. The DNA isolated from 5X 10^{7} cells was redissolved in 5 ml of lysis buffer and was mixed with 3 ml of the chloroform-iso-amylalcohol (3:1) mixture. Then centrifugation was conducted at 3,000 rpm for 20 min, the upper DNA-containing

layer was carefully removed and re-suspended in ½ volume of the chloroform-iso-amyl alcohol (3:1) mixture. After this centrifugation was conducted again at 3,000 rpm for 20 min and the supernatant was removed. NaCl was added to a final concentration 5% together with two volumes of cold ethanol. The precipitated DNA was wahed twice in 70% ethanol, air-dried, and dissolved in 10mM TE buffer (pH 7.5). The optical density of DNA solution was measured at 260 nm and 280 nm, and the ratio OD 260 nm/280 nm was calculated and shown to be more than 1.8. Equal quantity of DNA was electrophoresed in 15 g/L agarose gels and stained with ethidium bromide (5ml/L) for 2hr at 80 V. Ladder formation of oligonucleosomal DNA fragmentation was detected under UV light.

Results and Discussion

Degradation of genomic DNA, due to activation ofendonuclease, is one of the early events of bacterial destruction. This phenomenon was tested for the ability of Glycosides and flavonoids compounds to induce the cleavage in whole cell's DNA fragmentation. Pathogenic bacteria like *E.coli, Staphylococcus aureus* and *Klebsiella pneumoniae* were exposed to glycosides, flavonoids of *Caesalpinia coriaria*, at 10, 20, 30, and 40 µg /ml concentration and incubated for 24 hours. *E.coli, Staphylococcus aureus* and *Klebsiella pneumonia* when treated with the glycoside and flavonoids extracts of *Caesalpinia coriaria* for 24 hours showed a marked DNA fragmentation, and no fragmentation was observed in untreated cells (Fig 1,2,3 & 4).

Fragmentation of genomic DNA of pathogenic bacteria by the antibacterials

Influence of glycosides and flavonoids of *Caesalpinia coriaria* – against the genomic DNA of pathogenic organism agar gel electrophoresis was performed. The results confirmed that the antibacterials can interact with DNA of the bacteria. This may be due to damage of cell wall membrane that caused leakage of cytoplasmic content from the cells.

The antibacterials also inhibited the respiration of cells by inhibiting the respiratory chain dehydrogenase as well. This compound may cause damage on the DNA, resulting in less of replication and mutation of base pair of pathogenic bacteria thereby inhibiting bacterial growth.

This study is simple and easy evaluation method of detection of DNA damage, repair well as death of bacterial cells, chromosomal DNA fragmentation may be direct or indirect outcome of bacterial death. In early studies, *E.coli* cells- DNA damage exposed to H_2O_2 or antibiotics was also detected (Jose et al, 1994).

In a study by Mori and colleagues showed that DNA synthesis was strongly inhibited by flavonoids in Proteus vulgaris, whilst RNA synthesis was most affected in *S.aureus* (Mori., 1987). The authors suggested that the B ring of flavonoids may play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases and that this may explain the inhibitory action on DNA and RNA synthesis (Mori., 1987). Ohemeng et al screened 14 flavonoids of varying structure for inhibitory activity against *Escherichia coli* DNA gyrase and for antibacterial activity against *Staphylococcus epidermis, S.aureus, E.coli, Typhimurium* and *Stemotrophomonas maltophilia* (Ohemeng et al., 1993). More recently Palper and colleagues reported that quercetin binds to the Gyr B subsunit of E.coli DNA gyrase and inhibits the enzymes ATPase activity(Palper et al., 2003).

Figure.1 DNA fragmentation induced by the glycosides and flavonoids from *C.coriaria*

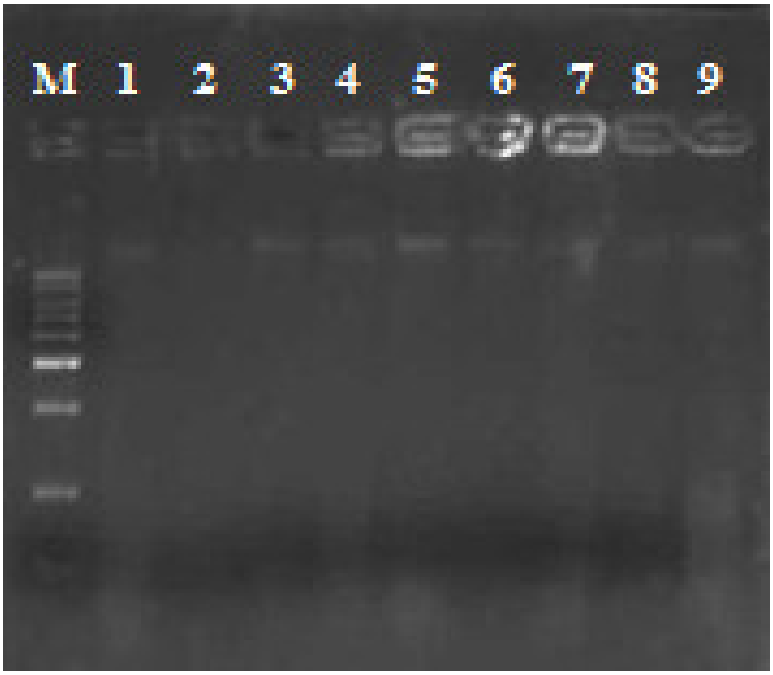


Figure.2 DNA fragmentation induced by the glycosides and flavonoids from *C.coriaria*

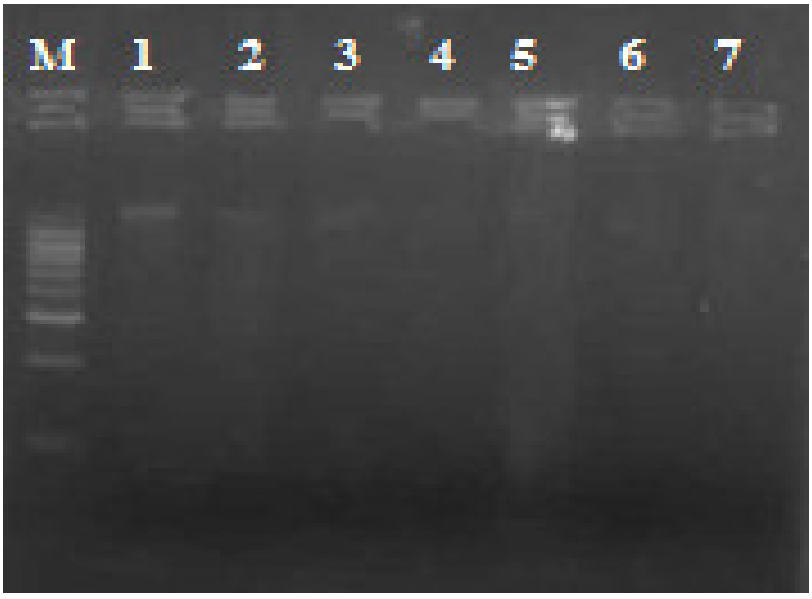


Figure.3 DNA fragmentation induced by the glycosides and flavonoids from *C.coriaria*

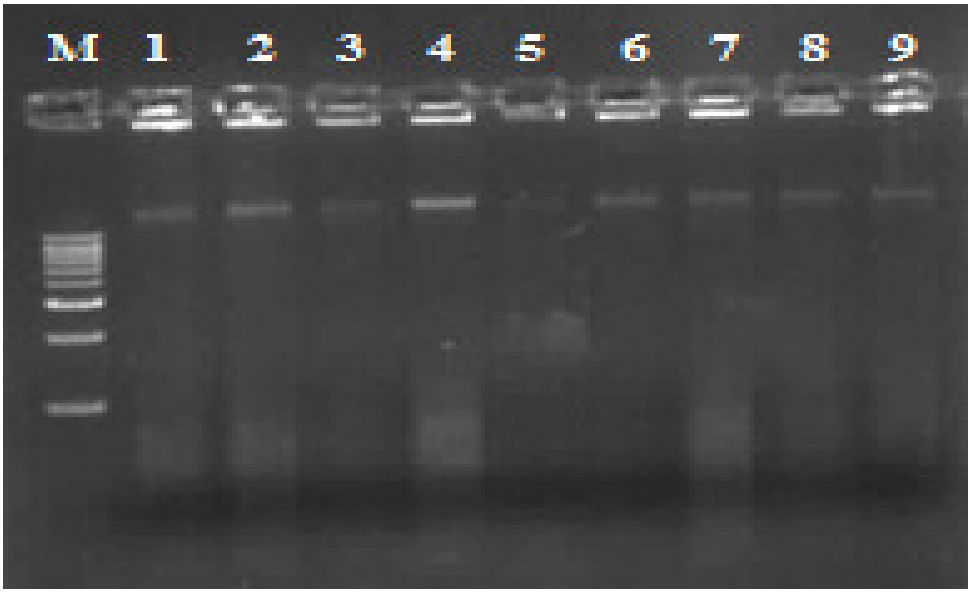
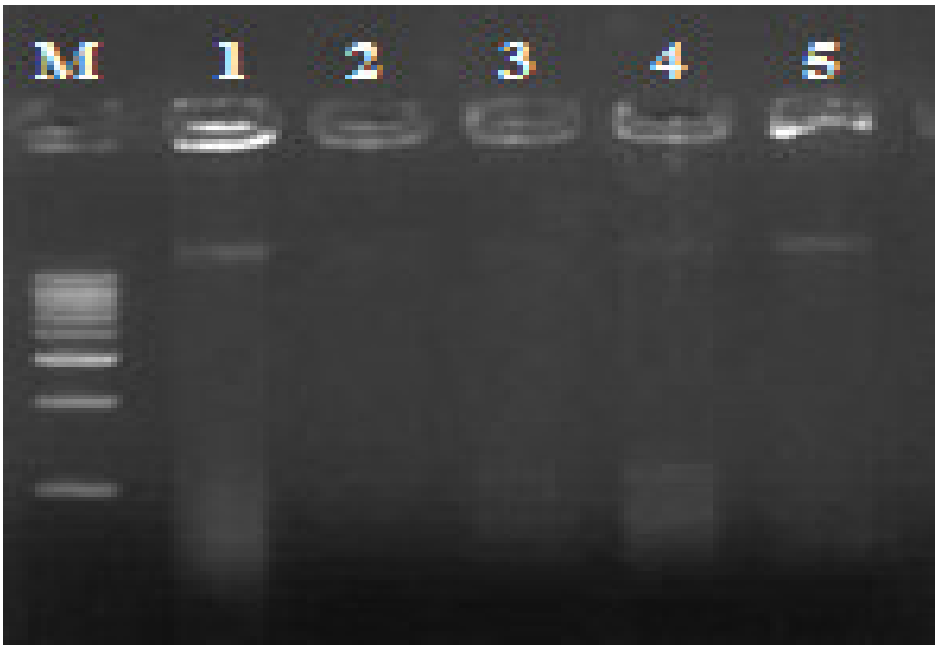


Figure.4 DNA fragmentation induced by the glycosides and flavonoids from *C.coriaria*



Enzyme binding was demonstrated by isolating E.coli DNA gyrase and measuring quercetin fluorescence in the presence and absence of the gyrase subunits. This work supports that Ohemeng et al and supports the suggestion that quercitin antibacterial activity against E.coli may be atleast partially attributable to inhibition of DNA gyrase.

To learn about a possible mechanism of antibacterial activity of the plant phytochemical flavonoids and glycosides from *C.coriaria* which caused a serve inhibition of *Escherichia coli, Methicillin resistant Staphylococcus aureus,* and *Klebsiella pneumoniae* in a dose- response manner? In conclusion it was evident that the glycosides and flavonoids interfere with the nucleic acid system at several different levels, thereby preventing the DNA from functioning as proper template.

Effect of flavonoids and glycosides from *C.coriaria* were found to be effective against *E.coli, S.aureus* and *K.pnuemoniae* at the concentration of 10-20 µg/ml. The identified compound is to be proceed for the structural analysis and can be recommended in the pharmaceutical industry.

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