INTERNATIONAL RESEARCH JOURNAL OF PHARMACY ISSN 2230 – 8407
Available online www.irjponline.com Research Article

DNA CLEVAGE STUDIES OF THE ETHANOLIC EXTRACTS OF THE BARK OF *BAUHINIA TOMENTOSA* L. AND THE WHOLE PLANT OF *MUSSAENDA FRONDOSA* L.

S. Gopalakrishnan* and E. Vadivel
Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627 012, India

Article Received on: 02/08/11 Revised on: 03/09/11 Approved for publication: 23/09/11

*Email: sgkmsu@yahoo.co.in

ABSTRACT
DNA cleavage studies of the ethanolic extracts of the bark of *Bauhinia tomentosa* L. (Fam, *Fabaceae*) and the whole plant of *Mussaenda frondosa* L. (Fam, *Rubiaceae*) have been performed. The cleavage of pUC18 DNA was evaluated by agarose gel electrophoresis. Both the plant extracts show a dose dependent increase from lower concentration to higher concentration. Ethanol extract of *Bauhinia tomentosa* L showed more cleavage activity than that of the *Mussaenda frondosa* L. The results have been compared with the standard DNA cleavage agent $FeSO_4$.
Keywords: DNA cleavage studies, *Bauhinia tomentosa* L, *Mussaenda frondosa* L, pUC18 DNA.

INTRODUCTION

Oxygen and its reactive species are very important in oxidative metabolism. They are produced in living cells by normal metabolism and by exogenous sources such as carcinogenic compounds and ionizing radiations[1]. The study of these reactive species and its effects on the organism have been of primary interest, since they induce some damage to cells by reacting with biomolecules such as proteins, lipids and cause serious lesions to DNA.

Reactive oxygen species (ROS)-induced oxidative DNA damage producing a variety of modifications at DNA level including base and sugar lesions, strand breaks, DNA-protein cross-link and base-free sites. However, DNA of all mammalian cells contains trace amounts of modified bases that are indicative of attack by oxidising species and they are removed by excision repairing enzymes, they are known to accumulate with age being associated with disease processes[2].

Designing drug for cleaving DNA is currently an area of considerable interest from chemical as well as biological stand points and offers potential applications in the field of medicine in the post-genomic era[3]. DNA cleavage is the process, which involved in various biological stages such as inflammation, mutagenesis, carcinogenesis and aging. As a consequence of clinical utility of DNA cleavage agents such as bleomycin, considerable effort has been made to identify and characterize naturally occurring molecules capable of cleaving the DNA[4], as such species may serve as lead structures for the development of novel anti-cancer drugs[5].

Mussaenda frondosa L. is one of the medicinally important plants belonging to the family *Rubiaceae,* commonly known as "Vellai ilai" in Tamil. Traditionally the leaves are used in the treatment of jaundice, asthma, hyperacidity, ulcers, leprosy, diuretic, wounds, swelling, fever and cough. They are also used as antimicrobial, diuretic, hypolipidemic, hepatoprotective agents. The plant *Bauhinia tomentosa* Linn. (Fam, *Fabaceae*) is commonly known as "Kanjana" in Tamil and "Phalgu" in Sanskrit, The dried leaves, buds and flowers are prescribed in dysentery[6]. The bruised bark is applied externally to tumors and wounds. A decoction of the root-bark is administered for inflammation of the liver and it is also used as a vermifuge. An infusion of the bark is also used as an astringent gargle. Leaf has the cytotoxicity and antioxidant activity, Flowers have anti-hyperglycemic and anti-lipidemic activity[7].

In the present work, the DNA cleavage studies of the ethanolic extracts of the bark of *Bauhinia tomentosa* L.and the whole plant of *Mussaenda frondosa* L. have been carried out.

MATERIALS AND METHODS

Collection of plants

Bauhinia tomentosa L. was collected in the month of September from Courtallam Hills of Tirunelveli District, Tamil Nadu, India. The whole plant of *Mussaenda frondosa* L.was collected in the month of October from Trivandrum, Western Ghats of South India, Kerala, India. The plants were identified by Prof. P. Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai, Tamil Nadu, India and the voucher specimens were deposited at Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

Preparation of plant powder and extract

The bark of *Bauhinia tomentosa* L. and the whole plant of *Mussaenda frondosa* L. were shade dried and pulverized to powder in a mechanical grinder separately. The powder of each plant (1 kg) was successively extracted with various solvents such as petroleum ether (40º- 60ºC), benzene, chloroform, ethanol, and water separately. The ethanol extracts of the bark of *Bauhinia tomentosa* L. and the whole plant of *Mussaenda frondosa* L. were used for the DNA cleavage studies.

DNA cleavage studies

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis[8,9]. Accurately weighed 300 mg of agarose was dissolved by boiling in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/l). The mixture was heated upto 55ºC then poured it into the cassette fitted with comb to solidify the gel. After the removal of the comb, gel placed in the electrophoresis chamber flooded with TAE buffer. DNA sample, extracts at different concentration, standard and DNA marker were loaded and 100 V of electricity passed till the dye front reaches the end of gel. The gel was then stained using ETBR solution (10 µg/ml) for 10-15 min and the bands under UV transilluminator was photographed.

RESULTS AND DISCUSSION

DNA cleavage is controlled by relaxation of supercoiled circular form of pUC18 DNA into nicked circular form and linear form[10]. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form. If one strand is cleaved, the supercoils will relax to produce a slower moving open circular form. If both strands are cleaved, a linear form will be generated. The gel electrophoresis experiments illustrates the cleavage of plasmid pUC18 DNA induced by the three binuclear complexes. DNA cleavage activity of the ethanolic extracts of the bark of *Bauhinia tomentosa* L. and the whole plant of *Mussaenda frondosa* L. are presented in Figure 1 and Figure 2.

Figure 1 shows that in the standard 5 mM $FeSO_4$, the DNA cleavage was complete. However, complete cleavage of the DNA was observed at 500 µg of the ethanolic extract of the bark of *Bauhinia tomentosa;* Lesser concentrations of the samples could not cleave the DNA.

The complete cleavage of DNA was observed with the standard 5 mM $FeSO_4$. From the Figure 2 it is evident that the ethanolic extract of the whole plant of *Mussaenda frondosa* L. showed increased partial cleavage activity with increase in the concentration of the samples. But even in the 500 µg concentration complete cleavage was not observed in this case.

CONCLUSION

From the present study, the traditional use of these two medicinal plants for wound healing have been validated by DNA cleavage studies

REFERENCES

1. Vertuani S, Angusti A, Manfredini S. The antioxidant and proantioxidants: an overview. Current Pharmaceutical Design 2004; 10:1677–1694.
2. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical induced damage to DNA: mechanisms and measurement. Free Radical Biology & Medicine 2002;32: 1102-1115.
3. Sambrook J, Fritsch E F, Maniatis T. Molecular cloning, A laboratory Manual. 2nd Edn, New York: Cold Spring Harbor Laboratory, Cold Spring Harbor; 1989.
4. Akhand A A, Kato M, Suzuki H, Miyata T, Nakashima I. Journal of Cellular Biochemistry 1998; 71: 243-253.
5. Clarke B, Cunningham D, Gallagher J F, Higgins H, McArdle P, McGinly J, Chochuin M, Sheerin D. Journal of Chemical Society 1994; 24:73.
6. Quriroga E N, Sampietro A R, Vattuone M A. Screening antifungal activities of selected medicinal plants, Journal of Ethnopharmacology 2010;74: 89-96.
7. Mannangatti V, Ayyasamy B, Rangasamy M, Emin B, Natesan S K. Antihyperglycemicand anti-lipidemic activity of ethanolic extract of Bauhinia tomentosa (linn) flower in normal and streptozotocin-induced diabetic rats, JournalGlobal Pharma Technology 2010; 2(3): 71-76.
8. Huang L, Wall M E, Wani M C, Navarro H, Santisuk T, Reutrakul V, Seo E K, Farnsworth N R, Kinghorn A D.Journal of Natural products 1996; 59**:** 290-292.
9. Miraj Kobad Chowdhury, Nazmul Ahsan and Anwarul Azim Akhand, Dhaka. Journal of Pharmaceutical Sciences 2009; 8(1): 75-79.
10. Ye-gao Chen, Li-na Hai, Ying Liu, and Xin-rong Liao., African Journal of Biotechnology 2009; 8(18): 4650-4652 .

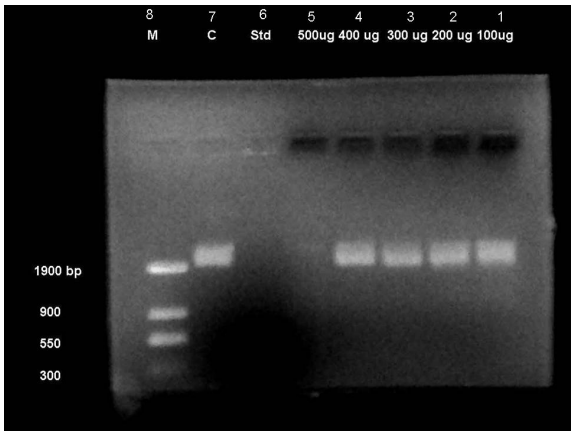

Figure 1. Agarose gel showing the results of electrophoresis of pUC18 DNA with EEBTB. Lane 1: pUC18 with 100 µg of EEBTB; Lane 2: pUC18 with 200 µg of EEBTB; Lane 3: pUC18 with 300 µg of EEBTB; Lane 4: pUC18 with 400 µg of EEBTB; Lane 5: pUC18 with 500 µg of EEBTB; Lane 6: pUC18 with 5 mM FeSO4 (standard); Lane 7: untreated pUC18 DNA (Control); Lane 8: Standard DNA molecular weight marker

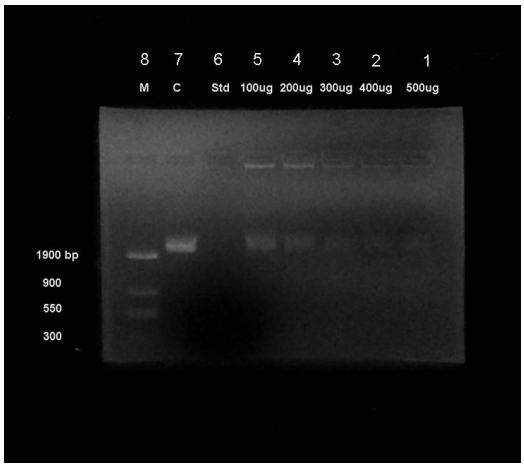

Figure.2. Agarose gel showing the results of electrophoresis of pUC18 DNA with EEMF. Lane 1: pUC18 with 100 µg of EEMF; Lane 2: pUC18 with 200 µg of EEMF; Lane 3: pUC18 with 300 µg of EEMF; Lane 4: pUC18 with 400 µg of EEMF; Lane 5: pUC18 with 500 µg of EEMF; Lane 6: pUC18 with 5 mM $FeSO_4$ (standard); Lane 7: untreated pUC18 DNA (Control); Lane 8: Standard DNA molecular weight marker

Source of support: Nil, Conflict of interest: None Declared