

## Original Research Article

# Restriction Inhibition Assay: A Qualitative and Quantitative Method to Screen Sequence Specific DNA Binder from Herbal Plants

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## Abstract

**Purpose:** To employ restriction inhibition assay (RIA) to screen phytochemical-rich fractions (PRFs) with high affinity for EcoRI and HindIII restriction sequences and correlate their interaction to an anticancer activity.

**Methods:** pBR322 linear plasmid DNA was used as a template to screen the sequence-selective inhibition of aqueous extracts of *Cinnamomum zeylanicum* and *Picrorhiza kurroa*, respectively. The template was further incubated with different concentrations of PRFs prior to digestion with restriction endonucleases HindIII and EcoRI. The Expressed Sequence Tags (ESTs) and Sequence Tag Sites (STS) of oncogenes were screened for the presence of EcoRI and HindIII restriction sequences to associate an anticancer property to PRF.

**Results:** The inhibitory concentrations of *Cinnamomum zeylanicum* aqueous extract against HindIII and EcoRI endonucleases were approximately 2.5 and 5 µg/µl, respectively. No binding was observed for *Picrorhiza kurroa* at both HindIII and EcoRI restriction sites. The saponin-rich fractions of *Cinnamomum zeylanicum* showed significant ( $p < 0.001$ ) inhibition as compared to control at concentrations of  $0.28 \pm 1.45$  µg/µl for EcoRI and  $0.11 \pm 2.68$  µg/µl for HindIII endonucleases. Both EcoRI and HindIII restriction sites were found repeatedly in the STS and ESTs of BRCA2, the early onset oncogene.

**Conclusion:** The inhibition of endonucleases by phytochemical-rich fractions provides direct evidence of the use of RIA for screening as well as demonstrating the binding specificity of these PRFs. The presence of 5'-AAGCTT-3' & 5'-GAATTC-3' in the ESTs of BRAC2 provides an insight into the use of screened components as leads in the search for novel anticancer compounds.

**Keywords:** Restriction endonucleases, Restriction sites, Phytochemicals, Restriction inhibition assay (RIA), Binding specificity, Oncogenes, Sequence tag sites, Expressed sequence tag, Anticancer.

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## INTRODUCTION

DNA is a primary target for many persuasive anti-tumor agents. Sequence specific binding of small molecules to DNA continues to attract considerable attention for developing effective therapeutic agents for control of gene expression

[1-6]. In recent years, there has been an inclination towards plant derived drug discovery due to its lesser side effect as compared to the chemically synthesized counterpart. This has generated great deal of interest in the development and screening of novel natural products of medicinal value from plant sources.

In the past, techniques such as DNA foot printing, *in vitro* transcription assay, restriction inhibition assay, absorption spectroscopy, fluorescence, circular spectroscopy were used to study the sequence specificity of anti-tumor drugs [7]. In this perspective, RIA showed more specificity for CA than GC-rich sequence for adriamycin and more affinity towards GC than AT-rich sequence for daunomycin [8], distamycin A showed more specificity for AT-rich sequence [9-10]. Moreover, RIA was found to be capable of evaluating the DNA-binding affinity of pyrrolo [2,1-c][1,4]benzodiazepine (PBD) antitumour antibiotics. The assay showed much more sensitivity in discriminating molecules of similar structure such as iso-Dc-81, DC-81 and neothramycin [11]. An assay has been proposed for rapid detection of *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase based on restriction endonuclease inhibition in many biological samples [12]. Recently, RIA was used to evaluate the rate of intercalator-driven platination of DNA [13] as well as binding specificity of mitoxantrone which showed more specificity for GC rich sequence [14].

Normally all these techniques were used to study the sequence specificity of drug-DNA interaction and none as used for screening a compound which binds to a specific sequence of DNA in a quantitative manner. In this outline we set out experiments to screen phytochemical rich fractions showing specificity for 5'-GC-3' and 5'-AT-3' sequences. Restriction inhibition assay was used to screen extract/phytochemicals showing affinity for *EcoRI* (5'-GAATTC-3') and *HindIII* (5'-AAGCTT-3') restriction sequences. Mitoxantrone and Distamycin-A were used as standard drugs interacting with *HindIII* and *EcoRI* restriction endonucleases. The current study is the first report to screen plant derived DNA binders from *Cinnamomum zeylanicum* bark and *Picrorhiza kurroa* rhizome extracts using RIA.

The clinical significance of the work is correlated to the anticancer potential of the phytochemicals by searching for 5'-GAATTC-3' and 5'-AAGCTT-3' in the STS and ESTs of BRCA 2 (oncogene). The STS are short tagged tracts of DNA sequence (200 to 500 base pair) that are operationally unique and have single occurrence in the human genome. Their location and base sequence are known and therefore they can be used as landmarks in genome mapping [15]. The ESTs are short sub-sequence of a transcribed cDNA sequence [16]. They may be used to identify gene transcripts, and are instrumental in gene discovery and gene sequence determination. Because these clones consist of DNA that is complementary to mRNA, the ESTs

represent portions of expressed genes [17]. The presence of 5'-GAATTC-3' and 5'-AAGCTT-3' sequences in the coding domain of an oncogene provides the necessary clinical relevance of the protocol to screen phytochemicals which bind to such sequences, thereby interfering with transcription and protein expression of an oncogene. This is central for antitumorogenesis of an antitumor drug.

## EXPERIMENTAL

### Plant material

*Cinnamomum zeylanicum* bark and *Picrorhiza kurroa* rhizome were gifts from Dr AN Nauriyal, Director of HNB Garhwal University, India. The plant materials were identified as per Auyervada literature, by a local expert in herbal gardens, and confirmed by Dr. HS Dhaliwal, Professor of Plant Biotechnology, Department of Biotechnology, Indian Institute of Technology, Roorkee, India.

### Drugs and chemicals

Mitoxantrone and distamycin-A were purchased from Sigma Aldrich Co., USA. Restriction Endonucleases *PvuII*, *HindIII* and *EcoRI* supplied with NEB (10 x) restriction buffer (100 mM Tris-HCl pH 7.5, 1 mM EDTA, 100 mM magnesium acetate, 500 mM potassium acetate) were purchased from New England Biolab, UK. Stock solution of distamycin-A (100  $\mu$ M), mitoxantrone (1 mM), aqueous extract of *Cinnamomum zeylanicum* and *Picrorhiza kurroa* (1 mg/ml) were prepared by dissolving each initially in minimum autoclaved milli-Q water before making up to volume with same. All solutions were stored at 4 °C.

### Screening of DNA-extract binding by RIA

The plasmid, pBR322, was linearized using *PvuII*. The linearized plasmid (600  $\mu$ g) was incubated with standard drugs-distamycin-A ( $\approx$  10  $\mu$ M), mitoxantrone ( $\approx$  220  $\mu$ M) and aqueous extract (10  $\mu$ g to 200  $\mu$ g per 20  $\mu$ l of reaction volume) for *Cinnamomum zeylanicum* and (10  $\mu$ g to 1000  $\mu$ g per 20  $\mu$ l of reaction volume) for *Picrorhiza kurroa* for 30 min at 37 °C. The reaction mixtures were further incubated with *EcoRI* and *HindIII* respectively for 1 hr at 37 °C in a final volume of 20  $\mu$ l. A control setup with the DNA along with each restriction enzyme was kept to analyze the results of the restriction inhibition assay. Each digestion was stopped by incubation for 20 min at 65 °C followed by 10 min at 4 °C.

### Preparation of the aqueous bark extract of *Cinnamomum zeylanicum*

Cinnamon bark (~500 g) was thoroughly powdered and kept sealed in cool, dry and dark conditions. Approximately 50 g was placed in a Soxhlet apparatus and extracted in aqueous medium for 60 h. The extract was concentrated in a rotary evaporator at reduced pressure to give a yield of 10 % which was then lyophilized (ScanVac CoolSafe Freeze Drying Tm) and used for further studies.

### Preparation of phytochemical-rich fractions (PRFs)

The aqueous extract of *Cinnamomum zeylanicum* which showed complete inhibition of restriction activity; was further fractionated into separate phytochemical enriched fractions using standardized protocol [18]. Four groups of phytochemicals were prepared as follows. (1) Anthraglycosides, bitter principles, flavonoids and arbutin (Fraction I): Powdered aqueous extract (1 g) is moistened with about 1 ml of 10 % ammonia solution; 5 ml methanol was added and the drug was then extracted for 10 min on a water bath. (2) Saponins (Fraction II): The methanol extract was prepared according to the method described for anthraglycosides. The extract was evaporated to about 1 ml, mixed 0.5 ml water and then extracted with n-butanol (saturated with water). (3) Cardiac glycosides (Fraction III): the powdered aqueous extract (1 g) was mixed with 5 ml of 50 % methanol and 10 ml of 10 % lead (II) acetate solution and then heated for 10 min over a hot water bath. The filtrate was cooled to room temperature and then extracted twice with 10-ml quantity of dichloromethane. The combined DCM extracts were evaporated, the residue dissolved in DCM-methanol (1:1) and used for further analysis. (4) Terpenes, coumarins, phenol carboxylic acids and valepotriates (Fraction IV): Powdered aqueous extract (1 g) was extracted by heating under reflux for 15 min with 10 ml DCM. The filtrate was evaporated to dryness, and the residue dissolved in 0.5 ml toluene.

The phytochemical rich fractions (I-IV) were further screened for inhibition with 5 2.5 µg/µl for *EcoRI* and *HindIII* endonucleases, respectively. The individual fractions were preincubated with linearized plasmid DNA (pBR322) at 37 °C for 30 min followed by addition of restriction endonucleases. Each digestion was stopped by incubation for 20 min at 65 °C followed by 10 min at 4 °C. Standard drugs mitoxantrone (≈ 220 µM) and distamycin-A (≈ 10 µM) were used as positive control for *HindIII* and *EcoRI* restriction inhibition activity, respectively. Each of the

samples was electrophoresed in a 1 % agarose gel at 100 V for 2 h.

### Screening for minimum inhibitory concentration (MIC) of active inhibitory PRFs

Each of fractions I, II and III were further screened for minimum inhibitory concentrations against both *EcoRI* and *HindIII* endonucleases. Each fraction was preincubated with linear DNA at 37 °C for 30 min followed by addition of restriction endonucleases. The samples were further electrophoresed in a 1 % agarose gel at 100 V for 2 hr.

### Screening for the presence of *EcoRI* and *HindIII* recognition sequence in mRNA, STS and ESTs of oncogene

The mRNA library of oncogene was screened for the presence of 5'-GAATTC-3' and 5'-AAGCTT-3' from Sanger.ac.uk/genetics/CGP/census. The oncogene with maximum hits of *EcoRI* and *HindIII* restriction sites were selected and analyzed further for the presence of 5'-GAATTC-3' and 5'-AAGCTT-3' in the STS and ESTs of the oncogene.

### Agarose gel electrophoresis

The digestion mixture (20 µl) was mixed with 5 µl of 6 x DNA loading dye (0.25 % bromophenol blue and 30 % glycerol) and loaded on 1 % horizontal agarose gel; which was run in Tris-acetate EDTA buffer (40 mM Tris base, pH 8.0, 18 mM glacial acetic acid and 1 mM EDTA) at 100 V for 2 h. The gel was exposed to 220 nm near UV region spectrum and then the DNA bands were visualized and analyzed for drug-DNA interaction studies. For quantitative determinations, the integrated intensity of the ethidium bromide fluorescence of the bands (relaxed form) were acquired and measured using DIANA and TINA programs (image analysis and acquisition system operating simultaneously with a Fuji camera connected to the PC; (Raytest, Isopenmeßgerä"te BmbH, Germany).

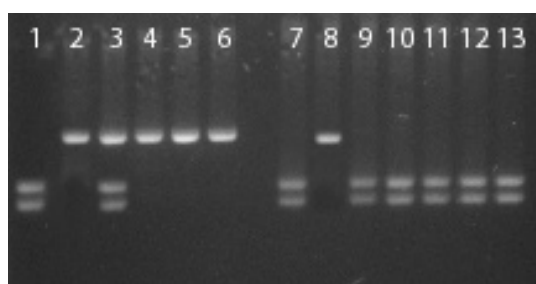
### Statistical analysis

Data are presented as mean ± SEM (standard error of mean) of three independent experiments. Statistical significance was evaluated by two tailed unpaired *t*-test using Origin software (Origin Lab Corporation, USA), version 6.1. *P* < 0.001 was taken as indicative of significant difference.

## RESULTS

### DNA-extract binding

DNA-extract binding was examined by measuring the inhibition of restriction endonucleases. Aqueous extract of *Cinnamomum zeylanicum* with concentrations ranging from 0.5  $\mu\text{g}$  to 50  $\mu\text{g}$  per  $\mu\text{l}$  and 0.5  $\mu\text{g}$  to 10  $\mu\text{g}$  per  $\mu\text{l}$  inhibited *EcoRI* and *HindIII* endonucleases, respectively, in a concentration-dependent manner. The MIC of the extract of *Cinnamomum zeylanicum* was approximately  $2.500 \pm 5.77$  for *HindIII* and  $5.006 \pm 9.28$   $\mu\text{g}/\mu\text{l}$  for *EcoRI* and was statistically significant ( $p < 0.001$ ) as compared to the positive control. While aqueous extract of *Picrorhiza kurroa* showed no binding with concentrations ranging from 0.5 to 50  $\mu\text{g}$  per  $\mu\text{l}$  for both *EcoRI* and *HindIII* restriction sequences. Mitoxantrone at  $113.828 \pm 0.4857$   $\text{ng}/\mu\text{l}$  or  $\approx 220$   $\mu\text{M}$  and distamycin-A at  $5.1797 \pm 0.0288$   $\text{ng}/\mu\text{l}$  or  $\approx 10$   $\mu\text{M}$  were bound selectively to *EcoRI* and *HindIII* restriction sites, respectively, and were used as positive control (Figures 1 and 2).

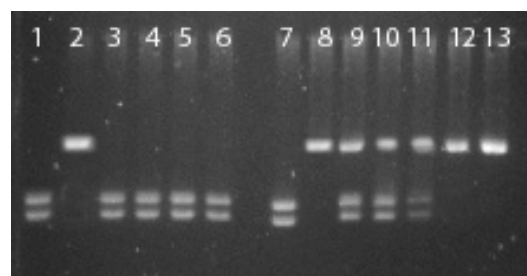


**Figure 1:** Agarose gel (1%) showing restriction inhibition assay of aqueous extract of *Cinnamomum zeylanicum* and *Picrorhiza kurroa* with *HindIII* restriction endonucleases. Lane 1: Linear plasmid (pBR322) + *HindIII*; Lane 2: Positive control-linear plasmid (pBR322) + *HindIII* + Mitoxantrone (220  $\mu\text{M}$ ); Lane 3-6: aqueous extract of *Cinnamomum zeylanicum*-linear plasmid complex was digested by *HindIII* with the extract concentration of 0.5, 2.5, 5 and 10  $\mu\text{g}/\mu\text{l}$ , respectively; Lane 7: Linear plasmid (pBR322) + *HindIII*; Lane 8: Positive control-Linear plasmid (pBR322) + *HindIII* + Mitoxantrone (220  $\mu\text{M}$ ); Lane 9-13: aqueous extract of *Picrorhiza kurroa*-linear plasmid complex was digested by *HindIII* with the extract concentrations of 0.5, 2.5, 5, 10 and 50  $\mu\text{g}/\mu\text{l}$ , respectively.

### RIA of PRF of *Cinnamomum zeylanicum* extract

The binding of fractions (I-IV) to both *EcoRI* and *HindIII* restriction sites were examined by inhibition of restriction endonucleases *EcoRI* and *HindIII*. Each of the Fractions (I, II and III) of *Cinnamomum zeylanicum* aqueous extract showed complete inhibition at approximately

$2.500 \pm 2.89$  and  $5.006 \pm 4.41$   $\mu\text{g}/\mu\text{l}$  for *HindIII* and *EcoRI* endonucleases, respectively. Fraction IV (terpenes, coumarins, phenol carboxylic acids, and valepotriates) showed no inhibition (Figure 3).



**Figure 2:** Agarose gel (1 %) showing restriction inhibition assay of aqueous extract of *Cinnamomum zeylanicum* and *Picrorhiza kurroa* with *EcoRI* restriction endonuclease. Lane 1: linear plasmid (pBR322) + *EcoRI*; Lane 2: Positive control-linear plasmid (pBR322) + *EcoRI* + Distamycin-A (10  $\mu\text{M}$ ); Lane 3-6: Aqueous extract of *Picrorhiza kurroa*-linear plasmid complex digested by *HindIII* with the extract concentration of 2.5, 5, 10 and 50  $\mu\text{g}/\mu\text{l}$ , respectively. Lane 7: Linear plasmid (pBR322) + *EcoRI*; Lane 8: Positive control-Linear plasmid (pBR322) + *HindIII* + Distamycin-A (10  $\mu\text{M}$ ); Lane 9-13: aqueous extract of *Cinnamomum zeylanicum*-linear plasmid complex digested by *EcoRI* with the extract concentrations of 0.5, 1.25, 2.5, 5 and 50  $\mu\text{g}/\mu\text{l}$ , respectively.



**Figure 3:** Agarose gel (1 %) showing restriction inhibition assay of fractions I to IV of *Cinnamomum zeylanicum* aqueous extract with both *EcoRI* and *HindIII* restriction endonucleases. Lane 1: Linear plasmid (pBR322) + *EcoRI*; Lane 2: Positive control-Linear plasmid (pBR322) + *EcoRI* + Distamycin-A (10  $\mu\text{M}$ ); Lanes 3-6: Fractions I, II, III and IV of *Cinnamomum zeylanicum* and linear plasmid complex digested by *EcoRI* with approximately 5  $\mu\text{g}/\mu\text{l}$ . Lane 7: Linear plasmid (pBR322) + *HindIII*; Lane 8: positive control-Linear plasmid (pBR322) + *HindIII* + Mitoxantrone (220  $\mu\text{M}$ ); Lane 9-12: Fractions I, II, III and IV of *Cinnamomum zeylanicum* and linear plasmid complex digested with *HindIII* by each fraction with approximately 2.5  $\mu\text{g}/\mu\text{l}$ .

### MIC of active inhibitory PRF (I to III) of *Cinnamomum zeylanicum*

The affinity of fractions I to III for *EcoRI* and *HindIII* restriction sequences were examined. Fraction I at concentrations of  $2.28 \pm 4.41$  and  $0.75 \pm 4.28$   $\mu\text{g}/\mu\text{l}$ , fraction II at  $0.11 \pm 2.68$  and

**Table 1:** A summary of quantitative and qualitative binding data for fractions I, II, III and IV of *Cinnamomum zeylanicum* extract

Drugs & Phytochemical Rich Fractions	DNA fragment length	Binding concentrations <sup>#</sup> (µg/µl)	Drugs sequence specificity identified*	Restriction enzyme used	Restriction enzyme restriction site
Mitoxantrone and distamycin-A (Positive control)	4.363 kb	113.828 ± 0.4857 ng/µl or ≈ 220 µM ( <i>HindIII</i> ), 5.1797 ± 0.0288 ng/µl or ≈ 10 µM ( <i>EcoRI</i> )	5'-AAGCTT-3' & 5'-GAATTC-3'	<i>HindIII</i> & <i>EcoRI</i>	5'-AAGCTT-3' & 5'-GAATTC-3'
Anthraglycosides, bitter principles, Flavonoids and Arbutin (Fraction I)	4.363 kb	2.28 ± 4.41 µg/µl* ( <i>EcoRI</i> ), 0.75 ± 4.28 µg/µl ( <i>HindIII</i> )	5'-AAGCTT-3' & 5'-GAATTC-3'	<i>HindIII</i> & <i>EcoRI</i>	5'-AAGCTT-3' & 5'-GAATTC-3'
Saponins (Fraction II)	4.363 kb	0.28 ± 1.45 µg/µl ( <i>EcoRI</i> ) <sup>‡</sup> , 0.11 ± 2.68 µg/µl ( <i>HindIII</i> ) <sup>‡</sup>	5'-AAGCTT-3' & 5'-GAATTC-3'	<i>HindIII</i> & <i>EcoRI</i>	5'-AAGCTT-3' & 5'-GAATTC-3'
Cardiac glycosides (Fraction III)	4.363 kb	0.748 ± 1.1547 ( <i>EcoRI</i> ) <sup>‡</sup> , 2.667 ± 1.764 µg/µl ( <i>HindIII</i> ) <sup>‡</sup>	5'-AAGCTT-3' & 5'-GAATTC-3'	<i>HindIII</i> & <i>EcoRI</i>	5'-AAGCTT-3' & 5'-GAATTC-3'
Terpenes, Coumarins, Phenol carboxylic acids, Valepotriates (Fraction IV)	4.363 kb	No binding observed	-----	<i>HindIII</i> & <i>EcoRI</i>	5'-AAGCTT-3' & 5'-GAATTC-3'

<sup>#</sup>Concentration of different phytochemical rich fractions required to inhibit restriction endonuclease cleavage by 100%.

\*Sequence selective binding of different phytochemical chemical rich fractions to the linear pBR322 plasmid DNA.

Data are the mean ± S.E.M of three independent experiment performed in triplicates; <sup>‡</sup>statistically significantly different compared to control ( $p < 0.001$ ).

**Table 2:** Occurrence of both *HindIII* (5'-AAGCTT-3') and *EcoRI* (5'-GAATTC-3') restriction sequences in the STS and ESTs of Breast cancer 2 early Onset Gene (BRCA 2).

NAME	BRCA 2
GENE ID	675
Occurrence of <i>EcoRI</i> (5'-GAATTC-3') in mRNA	5
Occurrence of <i>HindIII</i> (5'-AAGCTT-3') in mRNA	4
Occurrence of <i>EcoRI</i> (5'-GAATTC-3') in STS of BRCA 2	UniSTS-272209 (1)
Occurrence of <i>HindIII</i> (5'-AAGCTT-3') in STS of BRCA 2	UniSTS-272803 (1); UniSTS-272880 (1); UniSTS-505289 (1)
Occurrence of <i>EcoRI</i> (5'-GAATTC-3') in ESTs of BRCA	dbEST Id: 1457605; dbEST Id: 27576196; dbEST Id: 37888790; dbEST Id: 7737797;
Occurrence of <i>HindIII</i> (5'-AAGCTT-3') in ESTs of BRCA	dbEST Id: 6572441; dbEST Id: 37888790; dbEST Id: 22570003

0.28 ± 1.45 µg/µl and fraction III at 0.75 ± 1.15 and 2.67 ± 1.76 µg/µl inhibited *HindIII* and *EcoRI*, respectively, in a concentration-dependent manner (Table 1). The MIC of fraction II for both *HindIII* and *EcoRI* was comparatively lesser than that of the other two fractions I and III. The MIC obtained for each fraction was significantly different from that of control ( $p < 0.001$ ).

#### **EcoRI and HindIII recognition sequence in mRNA, STS and ESTs of the oncogenes**

The library of oncogenes was screened for the presence of 5'-GAATTC-3' and 5'-AAGCTT-3' sequence, respectively. The oncogene Breast

cancer 2, early onset mRNA (BRCA2) with maximum hits of *EcoRI* and *HindIII* restriction sites was selected (Table 1). The occurrence of 5'-GAATTC-3' and 5'-AAGCTT-3' in each STS and ESTs of the selected oncogene are shown (Table 2).

## **DISCUSSION**

Reagents that bind sequence selectively to double stranded DNA are of considerable interest in medicinal chemistry and molecular biology as they may possibly provide the tools for sequence specific modification of DNA and for

gene targeted drugs [1-6]. Even though DNA foot printing, *in vitro* transcription assay, absorption spectroscopy, fluorescence, circular dichromism spectroscopy have emerged as powerful tools for analyzing drug/DNA association phenomena [4], they have been unsuccessful in screening component binding specifically to nucleic acid sequences.

In this regard, restriction inhibition assay (RIA) yielded results similar to those obtained by DNA-binding affinity assays, namely, thermal denaturation and ethidium bromide displacement assays. Moreover, it was much more sensitive in discerning between molecules of comparable structures [11]. This assay was also used to determine the rates of intercalator-driven platination of DNA by four non-cross-linking platinum-acridine agents represented by the formula  $[Pt(am(2))LCI](NO(3))(2)$ , where am is a diamine nonleaving group and L is an acridine derived from the intercalator 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea (ACRAMTU) [12]. Restriction inhibition assay has also been used to measure  $O^6$ -alkylguanine-DNA alkyltransferase (AGT) levels in large numbers of small biological samples [13]. In this context, this assay can be performed in basic laboratories and would allow rapid processing of many samples concurrently, which could prove useful in clinical and epidemiological studies. Based on this perspective, the potential of RIA to screen drugs or phytochemical binding specifically to DNA is exceptional. The principle of RIA assay is based upon the ability of different phytochemical rich fractions to inhibit the cleavage activity of *HindIII* and *EcoRI* restriction endonucleases [14]. In the initial screening, aqueous extract of *Cinnamomum zeylanicum* showed inhibition of both *HindIII* and *EcoRI* endonucleases in a concentration dependent manner. The minimum inhibitory concentration (MIC) of the aqueous extract of *Cinnamomum zeylanicum* was  $2.500 \pm 5.77$  for *HindIII* and  $5.006 \pm 9.28$   $\mu\text{g}/\mu\text{l}$  for *EcoRI* but no binding was observed for aqueous extract of *Picrorhiza kurroa*.

The assay showed sequence specificity and affinity of the different fractions towards AAGCTT and GAATTC (reading in the 5'-3' direction). On the whole, the phytochemical rich fractions (PRFs) showed more affinity for *HindIII* restriction sequence than for *EcoRI* restriction sequence. The above observations support the hypothesis of using RIA to screen both qualitatively and quantitatively sequence specific DNA binding components from herbal plants.

The clinical significance of this novel screening protocol was evaluated by screening for the

presence of 5'-GAATTC-3' and 5'-AAGCTT-3' in the cDNA or mRNA of oncogenes from oncogene database of Sangers Laboratory, United Kingdom. The oncogenes BRCA 2 showed maximum repeats of 5'-GAATTC-3' and 5'-AAGCTT-3', respectively. To be more specific we analyzed the STS and ESTs, for the presence of 5'-GAATTC-3' and 5'-AAGCTT-3, respectively in the BRCA 2 early onset oncogene. The 5'-GAATTC-3' and 5'-AAGCTT-3 sequences were observed in the STS and ESTs of BRCA 2 cDNA/mRNA breast cancer 2, early onset oncogene. The STS are DNA sequences which are unique for a gene which may or may not be the coding region of the same [15,16]. On the other hand ESTs are unique coding sequences of any gene [17]. Therefore, the presence of drug specific sequence in the ESTs and STS may provide a unique target for cancer therapy. Consequently, targeting ESTs can directly present a site-directed attack to inhibit the expression of the oncogene by the screened phytochemical component.

## CONCLUSION

This study clearly shows that RIA can be used to screen compounds which can bind to specific sequences 5'-GAATTC-3' and 5'-AAGCTT-3 in the STS and ESTs of any oncogene. Therefore, the potential application of the findings of this study indicates the immense potential of the approach in screening novel plant derived inhibitor which binds to certain sets of sequences, and is unique for an oncogene.

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## REFERENCES

1. Maiti M, Kumar GS. Molecular aspects on the interaction of protoberberine, benzophenanthridine and aristolochia group of alkaloids with nucleic acid structures and biological perspectives. *Med. Res. Rev.*; in press; DOI 10.1002/med.20087.
2. Chaires JB. Drug-DNA interactions. *Curr. Opin. Struct. Biol.* 1998; 8: 314-320
3. Hurley LH. Secondary DNA structures as molecular targets for cancer Therapeutics. *Biochem. Soc. Trans.* 2001; 29: 692-696.
4. Dervan PB. Molecular recognition of DNA by small molecules. *Bioorg. Med. Chem.* 2001; 9: 2215-2235.
5. Neidle S. DNA minor-groove recognition by small-molecules. *Nat. Prod. Rep.* 2001; 18: 291-309.
6. Hurley LH. DNA and its associated processes as targets for cancer Therapy. *Nat. Rev. Cancer.* (2002); 2: 188-200.

7. Panousis C and Phillips D R. DNA sequence specificity of mitoxantrone. *Nucl. acids Res.* 1994; 22(8): 1342-1345.
8. Trist H, Phillips DR. In vitro transcription analysis of the role of flanking sequence on the DNA sequence specificity of Adriamycin. *Nucl. Acids Res.* 1989; 17(10): 3673-3687.
9. Chaires JB, Fox KR, Herrera JE, Britt M, Waring MJ. Site and sequence specificity of the daunomycin-DNA interaction. *Biochemistry.* 1987; 26(25): 8227-8236.
10. Farrow SM, Lee M, Souhami RL, Hartley JA. The effect of AT and GC sequence specific minor groove-binding agents on restriction endonuclease activity. *Chem. Biol. Interact.* 1995; 96: 125-142.
11. Puvvada MS, Hartley JA, Jenkins TC, Thurston DE. A quantitative assay to measure the relative DNA-binding affinity of pyrrolo[2,1-c][1,4]benzodiazepine (pbd) antitumor antibiotics based on the inhibition of restriction-endonuclease BamHI. *Nucleic Acids Res.* 1993; 21(16): 3671-3675.
12. Choudhury JR, Rao L, Bierbach U. Rates of intercalator-driven platination of DNA determined by a restriction enzyme cleavage inhibition assay. *J Biol Inorg Chem.* 2011 Mar;16(3):373-80.
13. Wilson BD, Strauss M, Stickells BJ, Hoal-van Helden EJ, van Helden PD. An assay for O6-alkylguanine-DNA alkyltransferase based on restriction endonuclease inhibition and magnetic bead separation of products. *Carcinogenesis.* 1994;15(10):2143-2148.
14. Hassan SA, Chauhan L, Barthwal R, Dixit A. A Qualitative and Quantitative Assay to Study DNA/Drug Interaction based on the Sequence Selective Inhibition of Restriction Endonucleases. *Trop J Pharm Res.* 2012; 11(5): 721-727.
15. Olson M et al. A common language for physical mapping of the human genome. *Science.* 1989 Sep 29; 245(4925):1434-1435.
16. Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, et al. Complementary DNA sequencing: expressed sequence tags and human genome project. *Science.* 1991; 252 (5013): 1651-1656.
17. Nagaraj SH, Gasser RB, Ranganathan S. "A hitchhiker's guide to expressed sequence tag (EST) analysis". *Brief. Bioinformatics.* 2007; 8(1): 6-21.
18. Wagner H, Blatt S, Zgainski EM. *Plant drug analysis.* Springer-Verlag, New York, Inc., 44 Hartz Way, Secaucus, NJ 07094-2491. 1984; p 320.