Inhibitory Effects of Bangladeshi Medicinal Plant Extracts on Interactions between Transcription Factors and Target DNA Sequences

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Several transcription factors (TFs) play crucial roles in governing the expression of different genes involved in the immune response, embryo or cell lineage development, cell apoptosis, cell cycle progression, oncogenesis, repair and fibrosis processes and inflammation. As far as inflammation, TFs playing pivotal roles are nuclear factor kappa B (NF-kB), activator protein (AP-1), signal transducer and activator of transcription (STATs), cAMP response element binding protein (CREB) and GATA-1 factors. All these TFs regulate the expression of pro-inflammatory cytokines and are involved in the pathogenesis of a number of human disorders, particularly those with an inflammatory component. Since several medicinal plants can be employed to produce extracts exhibiting biological effects and because alteration of gene transcription represents a very interesting approach to control the expression of selected genes, this study sought to verify the ability of several extracts derived from Bangladeshi medicinal plants in interfering with molecular interactions between different TFs and specific DNA sequences. We first analyzed the antiproliferative activity of 19 medicinal plants on different human cell lines, including erythroleukemia K562, B lymphoid Raji and T lymphoid Jurkat cell lines. Secondly, we employed the electrophoretic mobility shift assay as a suitable technique for a fast screening of plant extracts altering the binding between NF-kB, AP-1, GATA-1, STAT-3, CREB and the relative target DNA elements.

Keywords: AP-1 – CREB – GATA-1 – gene expression – medicinal plants – NF-kB – STAT-3 – transcription factors

Introduction

Transcription factors (TFs) play key roles in the regulation of gene expression (1). Accordingly targeting of TFs with DNA-based molecules (2,3) or low-molecular weight compounds (4,5) might be of great interest to develop selective molecules exhibiting therapeutic potential in several human pathologies (6–8). One of the best examples of the interplay between TFs, gene expression, drug design and discovery and novel therapeutic approaches is inflammation. Several TFs are indeed involved in inflammatory processes, including Nuclear Factor-kappa B (NF-kB) (9–14), activator protein 1 (AP-1) (15–17), signal transducer and activator of transcription (STATs) (18), cAMP response element binding protein (CREB) (19) and GATA-1 factors (20,21).
All these TFs have been described to play a significant role in regulating expression of genes coding pro-inflammatory cytokines and in the pathogenesis of a large number of human disorders, particularly those characterized by a chronic inflammatory component (17). These include rheumatoid arthritis (22,23), chronic asthma (24,25), diabetes mellitus type I (26) and Crohn’s disease (27).

With respect to anti-inflammatory activities, several plant-derived compounds exhibit significant effects (28–33). Therefore, medicinal plants represent a potential source of molecules of significant relevance for developing novel drugs, especially designed for treating and/or controlling chronic inflammatory conditions. Despite the therapeutic effects of medicinal plant extracts that are well-known and reviewed in several articles (34–44), sparse information is available on their effects on activity of TFs (45,46).

The main concern here was to screen Bangladeshi medicinal plants for their activity in inhibiting the interactions between nuclear factors and double stranded target oligonucleotides mimicking the NF-kB, AP-1, STAT-3, CREB and GATA-1 binding sites. To this aim, electrophoretic mobility shift assay (47) has been employed. Extracts from several medicinal plants have been considered, including Emblica officinalis, Aegle marmelos, Moringa oleifera, Terminalia arjuna, Vernonia anthelmintica, Oroxylum indicum, Saraca asoka, Rumex maritimus, Lagerstroemia speciosa, Red sandal, Cuscuta reflexa, Argemone mexicana, Hemidesmus indicus, Polyalthia longifolia, Cassia sophera, Paederia foetida, Hygrophilla auriculata, Ocimum sanctum and Aphanamixis polystachya.

**Methods**

**Plant Materials and Extraction**

The following plant material was extracted with absolute ethanol in cold extraction process: (i) the stem bark of C. sophera (yield = 10.22%), T. arjuna (yield = 10.1%), O. indicum (yield = 11.0%), A. marmelos (yield = 12.25%), S. asoka (yield = 14.16%), Red Sandal (yield = 13.2%), the dried leaves of O. sanctum (yield = 3.22%), the dried whole plant of C. reflexa (yield = 15.03%) and P. foetida Linn. (yield = 11.92%). The seeds of H. auriculata were extracted with 80% ethanol:water in cold extraction process (yield = 1.15%). The dried whole plant of L. speciosa was first defatted with petroleum ether and then extracted with chloroform and the yield was 7.1%. The roots of H. indicus Linn (yield = 8.9%), the stem barks of P. longifolia (Sonn.) (yield = 6.87%), the seed of V. anthelmintica (yield = 15.24) and the stem barks of A. polystachya Wall & Parker were extracted with 80% ethanol:water in cold extraction process. The extraction of A. polystachya resulted in two extracts, an oily phase and a solid mass, with a yield of 2.54% and 8.91%, respectively. The roots of M. oleifera Lamk. were extracted with absolute ethanol in a cold extraction process. This crude extract (yield = 14.54%) was further partitioned with n-hexane (yield = 7.39%), chloroform (yield = 5.94%), methanol (yield = 9.46%) and water (yield = 9.48%). The dried fruits of E. officinalis were extracted with absolute ethanol (yield = 9.33%). This ethanolic extract was defatted with petroleum ether and the defatted extract was successively fractionated with different solvent systems on the basis of polarity. The solvents were 100% dichloromethane, 25% ethylacetate-dichloromethane, 50% ethylacetatedichloromethane, 75% ethylacetate-dichloromethane, 100% ethylacetate, butanol and acetone and the remaining aqueous portions were separated. The dried whole plant of R. maritimus was first extracted with methanol (yield = 11.21%), then defatted with petroleum ether (yield = 9.87) and successively extracted with chloroform, ethylacetate and methanol (yield = 8.7). The whole plant of A. mexicana were extracted with methanol (yields = 1.25%).

**Cell Lines, Culture Conditions and Assays of in vitro Antiproliferative Activity**

Human erythroleukemia K562 (48), B lymphoid Raji (49) and T lymphoid Jurkat (50) cells were maintained in RPMI 1640 (Flow Laboratories, Irvine, UK) at 37°C in a humidified 5% CO2 atmosphere, supplemented with 10% fetal bovine serum (FBS; CELBIO, Milano, Italy), 100 units/ml penicillin and 100 μg/ml streptomycin (Aldrich, St.Louis, MA, USA). For analyzing the effects on in vitro cell growth, cells were seeded at the initial cell concentration of 30 000 cells/ml, cultured in the absence or presence of plant extracts and the cell number/ml determined using a model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL, USA) after 2, 3, 4, 5 days of cell culture. The IC50 values (i.e. the concentrations of extracts leading to 50% inhibition of cell growth, when treated cells are compared with untreated, control cultures) were determined in order to compare the activities of the different extracts. IC50 was determined usually after 4 days, when untreated cells are in the log phase of cell growth.

**Electrophoretic Mobility Shift Assay (EMSA)**

Electrophoretic mobility shift assay was performed by using double stranded 32P-labeled oligonucleotides as target DNA. Binding reactions were set up as described elsewhere in binding buffer (10% glycerol, 0.05% NP-40, 10 mM Tris-HCl pH 7.5, 50 mM NaCl, 0.5 mM DTT, 10 mM MgCl2), in the presence of poly(dI:dC),poly(dI:dC) (Pharmacia, Uppsala, Sweden), 2–5 μg of crude nuclear extracts and 0.25 ng of labeled
oligonucleotide, in a total volume of 20 μl (46,47). After 30 min binding at room temperature samples were electrophoresed at constant voltage (200 V for 1 h) through a low ionic strength (0.25 × TBE buffer) (1 × TBE = 0.089 M Tris-borate, 0.002 M EDTA) on 6% polyacrylamide gels until tracking dye (bromophenol blue) reached the end of a 16 cm slab. Gels were dried and exposed for autoradiography with intensifying screens at −80°C. In these experiments, DNA/protein complexes migrate through the gel with slower efficiency. In studies on the inhibitors of protein/DNA interactions, addition of the reagents was as follows: (i) poly(dI:dC),poly(dI:dC); (ii) labeled oligonucleotides mimicking the binding sites for TFs to be modulated; (iii) plant extracts; (iv) binding buffer; (v) nuclear factors. The nucleotide sequences of double-stranded target DNA utilized in these experiments were 5'-CGC TGG GGA CTT TTC ACG G-3' (sense strand, NF-kB), 5'-CGC TTG ATG AGT CAG CCG GAA-3' (AP-1), 5'-CAC TTG ATA ACA GGA AGT GAT AAC TCT-3' (GATA-1), 5'-CTG ATT TTC CCG AAA TGA CTT TCC ACG G-3' (CREB). The synthetic oligonucleotides utilized in this study were purchased from Sigma Genosys (Sigma Genosys, Cambs, UK). For quantitative determinations, the bands corresponding to protein-DNA complexes were analyzed using the BIO-RAD Gel Doc 2000 (Bio-Rad Genosys, Cambs, UK). For quantitative determinations, the complete set of results is shown in Table 1, which summarizes the data derived from four independent experiments. The highest antiproliferative activity was found using extracts from E. officinalis (IC₅₀ = 0.75 ± 0.04 and 0.51 ± 0.08 μg/ml from unfractionated and n-butanol extracts, respectively), O. indicum (IC₅₀ = 3.77 ± 0.32 μg/ml),

Results

Differential Inhibitory Effects of Extracts of Bangladeshi Medicinal Plants on in vitro Proliferation of Human Tumor Cell Lines

In order to obtain preliminary information on the biological properties of the employed extracts, their effects on cell growth were examined. The extracts isolated from E. officinalis, A. marmelos, M. oleifera, T. arjuna, V. anthelmintica, O. indicum, S. asoka, R. maritimus, L. speciosa, Red sandal, C. reflexa, A. mexicana, H. indicus, P. longifolia, C. sophera, P. foetida, H. auriculata, O. sanctum and A. polysychaca were first tested as potential inhibitors of in vitro proliferation of human erythroleukemic K562 cells. Figure 1 shows some representative results on the effects of increasing amounts of plant extracts on proliferation of K562 cells. The complete set of results is shown in Table 1, which summarizes the data derived from four independent experiments. The highest antiproliferative activity was found using extracts from E. officinalis (IC₅₀ = 0.75 ± 0.04 and 0.51 ± 0.08 μg/ml from unfractionated and n-butanol extracts, respectively), O. indicum (IC₅₀ = 3.77 ± 0.32 μg/ml),

Table 1. Antiproliferative effects of different extracts derived from Bangladeshi medicinal plants on erythroleukemic K562 cells

| Medicinal plant                                           | IC₅₀ (μg/ml) |
|-----------------------------------------------------------|-------------|
| E. officinalis (unfractionated extract)                   | 0.75 ± 0.04 |
| E. officinalis (n-butanol extract)                        | 0.51 ± 0.08 |
| A. marmelos (ethanol acetate fraction)                    | 135.50 ± 17.54 |
| A. marmelos (petroleum ether fraction)                    | 8.30 ± 0.99  |
| A. marmelos (tetrachloromethane fraction)                 | 30.25 ± 2.76 |
| M. oleifera                                              | 6.88 ± 6.05 |
| T. arjuna                                                 | 4.45 ± 0.50  |
| V. anthelmintica                                          | 66.84 ± 1.64 |
| O. indicum                                                | 3.77 ± 0.32  |
| S. asoka                                                  | 48.50 ± 12.02 |
| R. maritimus (aqueous fraction)                           | 39.94 ± 4.43 |
| R. maritimus (methanolic fraction)                        | 303.80 ± 118.44 |
| L. speciosa                                               | 46.00 ± 6.01 |
| Red sandal                                                | 36.94 ± 5.88 |
| C. reflexa                                                | 115.67 ± 47.14 |
| A. mexicana                                              | 59.56 ± 6.65 |
| H. indicus                                                | 626.76 ± 93.99 |
| P. longifolia                                             | 212.63 ± 20.04 |
| C. sophera                                                | 483.88 ± 134.42 |
| P. foetida                                                | 799.54 ± 44.49 |
| H. auriculata                                             | 523.77 ± 23.89 |
| O. sanctum                                                | 67.01 ± 2.24  |
| A. polysychaca (solid fraction)                           | 41.06 ± 0.02  |
| A. polysychaca (oil fraction)                             | 91.50 ± 17.76 |

The reported data represent the concentrations (average ± SD of four independent experiments) of plant extracts leading to a 50% inhibition of cell growth.
tumor cell lines. Figure 2 shows the effects on cell growth of Raji and Jurkat cells treated with increasing amounts of the extracts. Table 2 shows the IC50 values obtained for each cell line. The obtained data indicate that these plant extracts, despite some differences, inhibit the in vitro proliferation of all analyzed human tumor cell lines.

These results confirm and further extend the list of analyzed compounds previously published on the antiproliferative activity of E. officinalis (35), A. marmelos (37), V. anthelmintica, O. indicum, A. mexicana (39).

Identification of Plant Extracts Exhibiting High Levels of Inhibitory Activity on Protein-DNA Interactions

To determine whether inhibitory activities against TFs/DNA interactions are present in extracts from medicinal plants, the same plant extracts that were tested for antiproliferative activity were assayed for their ability in inhibiting the interactions between nuclear factors isolated from the human leukemic K562 cells and double stranded target oligonucleotides that mimic the binding sites of the NF-kB, AP-1, GATA-1, STAT-3 and CREB TFs.

Representative results are shown in Figs. 3–6 and summarized in Table 3. The electrophoretic mobility shift assay was performed using [γ-32P] S'-end-labeled oligonucleotides. The 5'-labeled oligonucleotides were incubated with plant extracts for 15 min and then nuclear extracts were added. After 15 min binding at room temperature, the samples were electrophoresed. The gels were dried and subjected to standard autoradiographic procedures.

As far as the inhibitory effects on NF-kB/DNA interactions (Figs. 3 and 4), the results show that high activity was exhibited by E. officinalis, ethyl acetate and petroleum ether fractions of A. marmelos, M. oleifera, V. anthelmintica, aqueous and methanolic fractions of R. maritimus, L. speciosa, H. indicus, P. longifolia, T. arjuna, S. asoka and solid and oil fractions of A. polystachya. On the contrary, high concentrations of extracts from O. indicum, Red sandal, C. reflexa, A. mexicana, C. sophera, P. foetida, H. auriculata and O. sanctum were unable to inhibit NF-kB/DNA interactions. In conclusion, when Figs 3 and 4 and Table 3 are considered together, the highest effects against NF-kB activity were displayed by T. arjuna, S. asoka and A. polystachya.

Terminalia arjuna, S. asoka and A. polystachya (solid fraction) are the most effective extracts inhibiting also AP-1/DNA interactions (Fig. 5A). Extracts showing a moderate inhibitory activity instead were derived from M. oleifera and aqueous fraction of R. maritimus. A lower activity was characterized by E. officinalis, A. marmelos, O. indicum, L. speciosa, H. indicus and oil fraction of A. polystachya. The other extracts were inactive.

| Medicinal plant | Raji cells (µg/ml) | Jurkat cells (µg/ml) |
|-----------------|---------------------|----------------------|
| E. officinalis (unfractionated extract) | 24.43 ± 9.4 | 33.28 ± 6.2 |
| E. officinalis (n-butanol extract) | 17.93 ± 8.0 | 10.77 ± 6.1 |
| A. marmelos (petroleum ether fraction) | 0.30 ± 0.1 | 35.25 ± 3.8 |
| M. oleifera | 1.82 ± 0.2 | 18.00 ± 2.0 |
| T. arjuna | 63.00 ± 5.0 | >500.00 |
| O. indicum | 23.20 ± 9.6 | 4.11 ± 0.1 |

The reported data represent the concentrations (average ± SD of four independent experiments) of plant extracts leading to a 50% inhibition of cell growth.

T. arjuna (IC50 = 4.45 ± 0.50 µg/ml), A. marmelos (IC50 = 8.30 ± 0.99 µg/ml, petroleum ether fraction) and M. oleifera (IC50 = 16.88 ± 6.05 µg/ml). The other tested plant extracts displayed lower antiproliferative activity (Table 1).

The most active plant extracts on K562 cells were also tested on B lymphoid Raji and T lymphoid Jurkat human
Terminalia arjuna was also the most active extract on inhibition of interaction between GATA-1 and DNA. As far as inhibition of GATA-1/DNA interaction, petroleum ether fraction of A. marmelos, R. maritimus aqueous fraction, S. asoka, A. polystachya solid fraction, M. oleifera, V. anthelmintica and L. speciosa extracts were efficient (Fig. 5B). Emblica officinalis extracts showed a intermediate activity, whereas 100 µg/binding reaction of extracts from A. marmelos, R. maritimus methanolic fraction, Red sandal, C. reflexa, A. mexicana, H. indicus, C. sophera, P. foetida, H. auriculata, O. sanctum and A. polystachya oil fraction were still unable to inhibit GATA-1/DNA interactions.

As far as STAT-3/DNA interaction, in addition to T. arjuna extracts the aqueous fraction of R. maritimus, S. asoka and solid fraction of A. polystachya extracts were also inhibitory (Fig. 6A), while only 75 µg/binding reaction of ethyl acetate fraction of A. marmelos and O. sanctum extracts displayed inhibitory activity. The remaining extracts were not capable of any activity.

Finally, regarding the CREB/DNA interaction studies, Table 3 and the related Fig. 6B show that, in addition to T. arjuna extracts, only those from S. asoka and E. officinalis possess an intermediate activity, whereas the other medicinal plant extracts are completely inactive.
Discussion

Inhibition of DNA-protein interactions could be a very promising strategy for the development of antitumor and antiviral therapeutic agents, as well as for the intervention in other different human pathologies related to inflammation, such as rheumatoid arthritis, chronic asthma and inflammatory bowel diseases (22–26).

DNA-binding drugs are extremely relevant and are the object of numerous research articles. Consistent results by our team and other research groups demonstrated that DNA-binding drugs inhibit the molecular interactions between DNA and TFs, leading to alteration of transcription (51–56). To analyze the possible interactions between TFs and the specific DNA elements we employed the EMSA for all preliminary experiments. In this context, several plant extracts were described able to inhibit DNA-protein interactions, and they may be a possible source of lead compounds that can alter gene expression.

The aim of this study was to screen several plant extracts for their ability to inhibit molecular interactions between DNA and TFs involved in the process of inflammation, including NF-κB, AP-1, STATs, CREB and GATA-1. Natural products derived from medicinal plants might play an interesting role for the identification of novel drugs for treating several diseases and they represent an important alternative source (57) for molecules inhibiting NF-κB activity.

For example, utilizing macrophages transfected with a vector coding for the luciferase reporter gene under the control of NF-κB cis-acting elements, investigators demonstrated that LPS (LipoPolySaccharide)-stimulated luciferase activity was affected by adding of extracts from the medicinal plant Glossogyne tenuifolia, utilized in Chinese medicine as a traditional antipyretic herb (58). Accordingly G. tenuifolia attenuates inflammatory mediator synthesis of activated macrophages through an NF-κB-dependent pathway. Moreover, the identified oleanolic acid and luteolin-7-glucoside, present in the extracts derived from this medicinal plant, inhibited LPS-stimulated inflammatory mediator production and NF-κB activation (58).

A second example is related to recent studies demonstrating that garcinol, a polyisoprenylated benzophenone derived from another medicinal plant, Garcinia indica, exhibits antioxidative effects in vitro. Liao et al. (59)
demonstrated with western blot analysis and gel-shift assays (EMSA) that garcinol strongly blocks the activation of eukaryotic TF NF-kB-induced by LPS. Moreover, for instance, transient transfection experiments showed that garcinol inhibited the NF-kB-dependent transcriptional activity.

The main conclusion of the present article is that inhibitory activity against TFs is displayed by several plant extracts, as shown in Table 3, in which the IC50 values (concentration leading to 50% inhibition of TFs/DNA interactions) of the electrophoretic mobility shift assays are summarized. The data suggest that *P. longifolia* represents an extract displaying interesting selectivity in inhibiting only the NF-kB/DNA interaction, whereas for example *T. arjuna* demonstrated a very high activity in all TFs/DNA binding experiments. Both extracts derived from *E. officinalis* interfered preferentially with NF-kB/DNA interaction with lower selectivity than *P. longifolia*, while only one fraction of *A. marmelos* (pet. ether extract) was selective for GATA-1/DNA interaction. *Hemidesmus indicus* also appears to be an interesting selective extract, since it inhibits NF-kB/DNA interaction at 12μg/ml, while it is active on the other TF/DNA interactions only when added at 50μg/ml or higher concentrations.

Both *A. polystachya* fractions proved a high, but not selective, activity particularly on the inhibition of NF-kB/DNA interactions. *Moringa oleifera* and *L. speciosa* extracts were active on NF-kB, AP-1 and GATA-1 and inactive on other TFs. *Vernonia anthelmintica* demonstrated activity with NF-kB and GATA-1 only. *Saraca asoka* inhibited all TFs/DNA interactions even if at different concentrations. The aqueous fraction of *R. maritimus* was more active in inhibiting NF-kB/DNA interaction than the methanolic extract, which interfered at intermediate concentrations with NF-kB/DNA interaction only. *Ocimum sanctum* was the only extract that selectively inhibited the STAT-3/DNA interaction. Finally, the remaining medicinal plant extracts were ineffective on all protein/DNA interactions. These data support the concept that medicinal plant extracts represent a potential source of compounds exhibiting the ability to suppress the molecular interactions between TFs and target DNA sequences. Further experiments (Gas Chromatography/Mass Spectrometry (GC/MS), High-Performance Liquid Chromatography/Mass
Spectrometry (LC/MS) should be performed to identify pure compounds exhibiting these activities. However, it should be pointed out that several compounds have already been described in plant extracts analyzed in the present article. For instance, three thiocarbamate (TC)- and isothiocyanate (ITC)-related compounds have been isolated from the leaves of *M. oleifera* (60). In addition, the oleanane triterpenes arjunic acid, arjungenin and their glucosides, arjunetin and arjunglucoside II, were isolated from the bark of *T. arjuna* (61). Several flavonoids including 2',3,4,4'-tetrahydroxychalcone, 5,6,7,4'-tetrahydroxylavone and butin, were separated from the seeds of *V. anthelmintica* (62). The leaves of *L. speciosa* afforded a new natural product, 31-norlargerenol acetate, along with known compounds 24-methylenecycloartanol acetate, its 31-nor analog, largerenol acetate, tinotufolins C and D, lutein, phytol, sitosterol and sitosterol acetate (63). Pyrogallol was identified in extracts from *E. officinalis* (35).

Therefore, EMSA analysis of the activity of the compounds already demonstrated to be present in the plant extracts exhibiting inhibitory activity on TF/DNA interactions is expected to help in identifying the molecules responsible for the activity of the plant extracts.

As far as the possible relationships between effects on cell growth and effects on protein-DNA interactions, when data from Tables 1–3 are compared, evidently some extracts, including for example *O. indicus*, *E. officinalis*, *T. arjuna* and *A. marmelos* (petroleum ether fraction), inhibit TFs/DNA interactions and are also highly effective in inhibiting cell proliferation. This is expected, considering the relevant involvement of TFs on cell cycle progression. On the contrary, other extracts, even inhibiting protein/DNA interactions, do not display antiproliferative activity.

Therefore, our study suggests that medicinal plant extracts exhibiting inhibitory activity on cell proliferation

**Table 3. Inhibitory effects of plant extracts on the interactions between nuclear factors and double stranded synthetic oligonucleotides mimicking the binding sites of the NF-κB, AP-1, GATA-1, STAT-3 and CREB transcription factors**

| Medicinal plant | NF-κB* | AP-1* | GATA-1* | STAT-3* | CREB* |
|-----------------|--------|-------|---------|---------|-------|
| *E. officinalis*|        |       |         |         |       |
| unfractionated extract | 12.5 | 25 | 50 | 100 | 50 |
| n-butanol extract | 12.5 | 25 | 50 | 100 | 100 |
| *A. marmelos* |        |       |         |         |       |
| ethyl acetate fraction | 25 | 100 | >400 | 75 | >100 |
| petroleum ether fraction | 50 | 50 | 6.25 | >100 | >100 |
| CCl₄ fraction | >100 | >400 | >400 | >100 | >100 |
| *M. oleifera* |        |       |         |         |       |
| 3.12 | 3.12 | 3.12 | 1.50 | 6.25 | |
| *T. arjuna* |        |       |         |         |       |
| >100 | 50 | 100 | >100 | >100 | >100 |
| *V. anthelmintica* |        |       |         |         |       |
| 1.5 | 6.25 | 12.5 | 12.5 | 12.5 | |
| *O. indicum* |        |       |         |         |       |
| 125 | 125 | 125 | 6.25 | 100 | >100 |
| *R. maritimus* |        |       |         |         |       |
| acqueous fraction | 12.5 | 12.5 | 12.5 | 6.25 | >100 |
| methanolic fraction | 25 | 100 | 400 | >100 | >100 |
| *L. speciosa* |        |       |         |         |       |
| 25 | 50 | 25 | >100 | >100 | >100 |
| *Red sandal* |        |       |         |         |       |
| 100 | 400 | 400 | >100 | >100 | >100 |
| *C. reflexa* |        |       |         |         |       |
| >100 | 400 | 400 | >100 | >100 | >100 |
| *A. mexicana* |        |       |         |         |       |
| 100 | >400 | >400 | 100 | >100 | >100 |
| *H. indicus* |        |       |         |         |       |
| 12.5 | 50 | >100 | >100 | >100 | >100 |
| *P. longifolia* |        |       |         |         |       |
| 12.5 | >100 | >100 | >100 | >100 | >100 |
| *C. sophera* |        |       |         |         |       |
| 100 | >100 | >100 | >100 | >100 | >100 |
| *P. foetida* |        |       |         |         |       |
| >100 | 100 | 100 | >100 | >100 | >100 |
| *H. auriculata* |        |       |         |         |       |
| >100 | >100 | >100 | >100 | >100 | >100 |
| *O. sanctum* |        |       |         |         |       |
| >100 | >100 | >100 | 75 | >100 | >100 |
| *A. polystachya* |        |       |         |         |       |
| solid fraction | 0.75 | 6.25 | 12.5 | 12.5 | 100 |
| oil fraction | 6.25 | 50 | >100 | 50 | >100 |

*Concentrations are expressed in μg/binding reaction (20 μl). The data reported represent the concentrations of plant extracts leading to a 50% inhibition of protein/DNA interactions.*
should undergo analysis for possible antitumor activity, while extracts displaying inhibition of TFs/DNA interactions without effects on cell growth kinetics might be employed to control TFs-dependent gene expression without cytotoxic effects, including the case of inflammatory processes involved in relevant human pathologies, such as rheumatoid arthritis and cystic fibrosis.

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