Journal of Traditional and Complementary Medicine 5 (2015) 119–126

HOSTED BY

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Review article

# Creosote bush lignans for human disease treatment and prevention: Perspectives on combination therapy

John Gnabre [a], Robert Bates [b], Ru Chih Huang [c, *]

[a] Mal-4 Research Institute, Baltimore, MD, USA
[b] Department of Chemistry, University of Arizona, Tucson, AZ, USA
[c] Department of Biology, Johns Hopkins University, Mudd Hall, Baltimore, MD, USA

ARTICLE INFO

*Article history:*
Received 25 September 2014
Received in revised form
2 October 2014
Accepted 13 November 2014
Available online 12 March 2015

*Keywords:*
cancer
creosote bush
lignans
nordihydroguaiaretic acid
viruses

ABSTRACT

The medicinal properties of the most successful plant in the deserts of the western hemisphere, the creosote bush (*Larrea tridentata*), are evidenced by the long traditional usage of the plants by the Native Americans Indian tribes in Southwestern North America and the Amerindians from South America. The plant is rich in simple bisphenyl lignans and tricyclic lignans known as cyclolignans. These compounds are responsible for many of the pharmacological activities of extracts of the plants. Some of these activities, namely antiherpes, antioxidant, antifungal, and anti-inflammatory, were known a century ago. Only recently have further studies revealed other crucial activities of the same plant molecules as powerful agents against human immunodeficiency virus, human papillomavirus, cancer, neurodegenerative diseases, and symptoms of aging. Molecular mechanisms underlying the antiviral and anticancer activities have been elucidated and involve the inhibition of SP1 dependent gene transcription. This review summarizes the recent findings on creosote bush lignans. We introduce the concept of a cocktail of safe well-characterized natural products from the creosote bush that would represent a bridge between oriental herbal medicines and Western drug-based therapies.


## 1. Introduction

Medicine has been intimately associated with human history since the dawn of civilization. The use of herbal concoctions for medicinal purposes dates back at least to several millennia BC from the records of ancient Egyptian, Chinese, Babylonian, Greek, and Roman cultures.[1] To date < 2% of botanical resources have been exploited for bioactive molecules. These have resulted in nearly 25% of all prescription medicines, which underscores the vital importance of investigating thoroughly the plant kingdom for potential treatment modalities. Nature is the first, and for millions of people, the only source for medicines. According to reports of the World Health Organization (WHO), 80% of the world population depends on herbal medicine for primary health care.[2] In spite of scientific progress in organic synthesis and biochemistry over the past century, current medicine still relies primarily on drugs isolated from plants, fungi, marine botanicals, molds, etc. to save countless human and animal lives. Although some of these molecules have served as templates for semisynthetic congeners currently in clinical use, what nature designed often cannot be easily duplicated.

Medicinal lignans have aroused the interest of the medical community only over the past 2 centuries with the discovery of the medicinal properties of the highly cytotoxic compounds podophyllin and podophylotoxin (Fig. 1). Podophyllin is a resinous powder extracted primarily from the rhizomes of American Mayapple (*Podophyllum peltatum*) and Himalayan Mayapple (*Podophyllum hexandrum* or *emodi*). Podophyllotoxin, also known as podofilox, is extracted from the roots and rhizomes of several *Podophyllum* species. These compounds are powerful antineoplastic agents.[3]

*Podophyllum*-derived compounds, along with other complex lignans, are part of two important classes of lignans known as aryltetralin lignans and arylnaphthalene lignans (Fig. 1). Current efforts are aimed at using these molecules as templates for drug discovery. Both aryltetralin and arylnaphthalene lignans serve as lead compounds for drug development as they exhibit a plethora of

* Corresponding author. Department of Biology, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA.
*E-mail address:* rhuang@jhu.edu (R.C. Huang).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2014.11.024

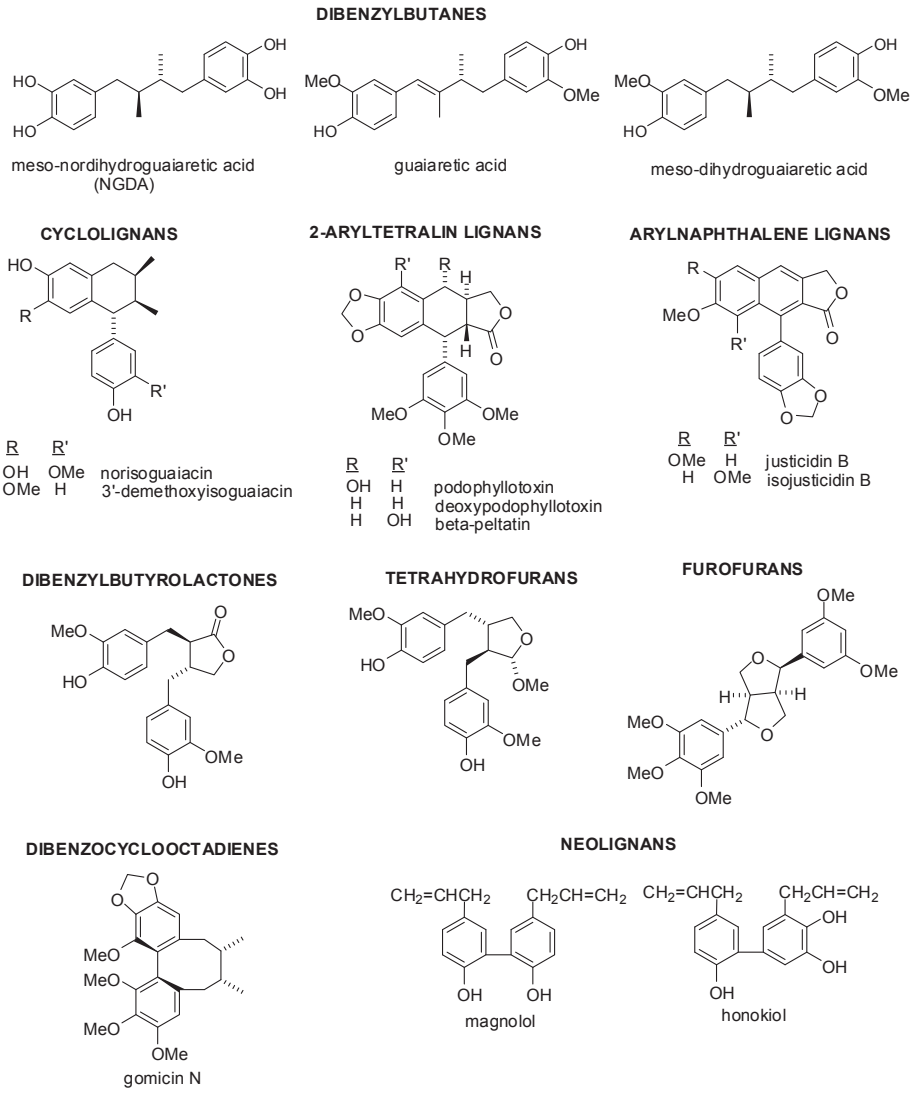


**Fig. 1.** Major classes of lignans with prototype molecules. The dibenzylbutanes or linear lignans and the cyclolignans are found in most species of *Larrea* including the creosote bush.

biological activities.[4] Other equally complex lignans have been isolated from the Chinese mock-barberry, *Schisandra chinensis* (五味子 wǔ wèi zǐ). This plant is the source of numerous benzocyclooctadiene lignans that support the uses of the plant extract in traditional oriental medicine.[5] Several classes of lignan are shown in Fig. 1, where the complexity of the molecules of the other classes sharply contrasts with the structural simplicity of the dibenzylbutane or linear type lignans found in the creosote bush.

## 2. Distribution and botanical description of the creosote bush

The creosote bush, *Larrea tridentata* (DC.) Cov., is a desert shrub also known as gobernadora (meaning *lady governor* to reflect the shrub's remarkable ability of retaining water by inhibiting, through its roots, the growth of most nearby plants), hediondilla (Spanish for little stinker), and guamis. The creosote bush is a widespread perennial flowering bush thriving in the deserts of the Southwestern USA and south through the New World deserts. Its generic name *Larrea* honors the Spanish scientist J. A. Hernández Pérez de Larrea, who first described the species.[6,7]

It is believed that the creosote bush originated in South America and spread to North America some millennia ago. In some arid regions of southwestern USA, the plant not only thrives as the most dominant vegetation but has also genetically adapted as evidenced by genotypes that vary according to regions. The plant grows in all desert regions, but only the species that grow in South America and in North America's largest desert (the Chihuahuan) carries two sets of chromosomes in each cell nucleus. In the Sonoran desert, where winters are mild and rains fall during both the summer and winter, the plant has four sets of chromosomes. By contrast, in the Mojave desert, the smallest and driest desert of the USA, where summers are very long and hot, the plant is hexaploid.[7,8] Whether the chromosomal variations are in response to the climate of the region or correlate with the quantity and diversity of the production of secondary metabolites is unknown. There is considerable pharmacologic interest in these metabolites, which the plant appears to use for defense.

## 3. Use in traditional and complementary medicine

The wide range of pharmacological activities ascribed to the creosote bush is evidenced by the myriad of traditional uses of the plant extract among various indigenous societies. Pima and Maricopa Indians use the extracts or decoctions of the shrub for a wide

variety of human ailments including chicken pox, tuberculosis, sexually transmitted diseases, menstrual pain in women, and snake bites. Other traditional uses of the leaves include treatment of cold virus infections, influenza, diabetes, skin sores, arthritis, sinusitis, gout, anemia, fungal infections, and cancer. Usages of the plant for antimicrobial properties, women's premenstrual symptoms, allergies, autoimmune diseases, and rheumatism are still of common occurrence in several indigenous cultures. Chaparral tea (made from creosote bush) is commonly used to treat gallbladder and kidney stones. A thorough review of the medicinal uses of creosote bush is available.[6] This report may not be exhaustive, as evermore compounds are discovered from *Larrea* species and newer ethnobotanical applications are described.

## 4. Phytochemistry

Lignans are one of the most important classes of natural products in *Larrea* species. They include nordihydroguaiaretic acid (NDGA) and its methylated derivatives. These compounds were reported to occur in the desert plant several decades ago.[9] Later, cyclolignans and furanoid lignans were added to the list.[10] The plant resin has been reported to contain a total of 19 flavonoid aglycones, some flavonoid glycosides, and a large quantity of essential oils (about 300 volatile compounds) making up 0.1% of the dry weight and containing 67 nonvolatile compounds.[6] Along with these compounds, creosote bush extracts contain saponins, sapogenins, tannins, sterols, monoterpenes, and sesquiterpenes. The list of creosote bush ingredients is continuously being updated, as evermore state-of-the-art analytical instruments with high-throughput screening are used to study the plant constituents.

## 5. Lignans

Lignans were first defined by Haworth et al[11] as dimers of phenylpropanoid (C6-C3) units linked by the central carbons of their side chains. The early lists of lignans have been updated by a compilation of > 350 new lignans discovered since 2005.[12] Lignans are divided into subclasses based on the way the oxygen atom is incorporated in the skeleton and the cyclization of the molecule.[13] Below we list the subclasses for this important class of natural products. Their chemical structures are depicted in Fig. 1 with prototype molecules.

### 5.1. Linear lignans (dibenzylbutanes, NDGA derivatives)

This class consists of NDGA and its derivatives. NDGA, one of the major constituents of creosote bush, makes up to 50% of the resin covering the surface of the leaves. From 5 g to 10 g of NDGA can be extracted from 100 g of the dry leaves. This exceptional yield accounts for the use of creosote bush as a primary commercial source for NDGA. NDGA is accompanied by many O-methylated derivatives, such as guaiaretic acid, which is the main constituent of the heartwood resin of *Guniacum oficinule*.[11,14]

### 5.2. Dibenzylbutyrolactones

The common wood constituent matairesinol is a dibenzylbutyrolactone. This subtype has not been reported in creosote bush. Lactone-containing molecules exhibit a greater antitumor potency. However, lactones can be easily metabolized in the gastrointestinal (GI) tract by esterases, which deactivate the molecule.

### 5.3. Cyclolignans

Norisoguaiacin and its 3′-methyl derivative are examples of cyclolignans. These two lignans occur in creosote bush in < 0.1% and were found to be significantly more active in an anti-human immunodeficiency virus (anti-HIV) assay than NDGA and its methylated derivatives.[15]

### 5.4. Tetrahydrofurans

Substituted tetrahydrofuran lignans can be categorized into three subtypes: 2,5-diaryltetrahydrofurans, 2-aryl-4-benzyltetrahydrofurans, and 3,4-dibenzyltetrahydrofurans.[12]

### 5.5. Furofurans

This is one of the major subclasses of the lignan family. These lignans exhibit a wide range of biological functions including antitumor activity,[16] and have been reviewed.[12]

### 5.6. Dibenzocyclooctadienes

Gomisin N was isolated from *Schisandra chinensis* (五味子 wǔ wèi zǐ). It is the most active dibenzocyclooctadiene, with antiproliferative activity in human colorectal carcinoma.[17] *Schisandra* species are rich in benzocyclooctadiene lignans with a plethora of biological activities. Their pharmacology has been the subject of an exhaustive review.[5]

### 5.7. Neolignans

Classic lignans are phenylpropane dimers linked by a bond between positions C8 and C8′, while neolignans are those dimers whose coupling patterns differ from such a C8–C8′ linkage. Examples include magnolol and honokiol, principal constituents of the Asian medicinal plant *Magnolia obovata*. A number of pharmacological activities such as sedation, antistress, anticancer, anti-inflammatory, and hepatoprotective effects associated with the magnolia plant extract are due in part to these two neolignans.[18] With the discovery of new structures, neolignans have been divided into 15 subtypes, showing the diversity of compounds in this class. Some of the constituents include biphenyl derivatives, eupomatenoid, benzofurans, burchellin benzofurans, bicyclooctanes, and futoenones.[12] It is beyond the scope of this review to cover these subclasses although they are clinically relevant in the fight against human diseases such as cancer, HIV/AIDS, and neurodegenerative diseases. In this review, we will focus on the simple NDGA type diphenylbutane lignans of creosote bush.

## 6. Pharmacological activities of creosote bush lignans

### 6.1. NDGA, the major metabolite and potent antioxidant

The biology and chemistry of the constituents of *Larrea* species including its major metabolite NDGA have been the subjects of excellent reviews.[9,19] Although useful as an antioxidant, NDGA possesses an inherent toxicity and was withdrawn from use by the United States Food and Drug Administration in 1968 for causing cystic nephropathy in rats. High doses of the plant extract were shown to cause hepatotoxicity, dermatitis, biliary toxicity, and other ailments, in part as a result of NDGA effects.[20] Indeed, NDGA possesses intrinsic hepatoxicity as the molecule is metabolized by the liver into O-methyl and glutathione conjugates.[20] This toxicity is virtually eliminated when NDGA is O-methylated. For this review, we present the various findings on the less toxic methylated

derivatives of NDGA, which have been shown to possess remarkable antiviral and anticancer activities.

### 6.2. Methylated derivatives of NDGA

#### 6.2.1. Antiviral activities

Methylated derivatives of NDGA, such as 3′-O-methyl-NDGA (Mal-4), and other lignans with various degrees of O-methylation, were first shown to possess activity against HIV in the mid-1990s.[15] Mal-4 is a yellowish oil that is highly active and readily permeates cell membranes and viral coat layers. Likewise, the dimethoxy and trimethoxy derivatives are oily and active. These activities were also observed with O-methylated cyclolignans isolated from the same plant extract. Unlike NDGA, all these compounds were effective at inhibiting HIV replication in cell cultures (laboratory strains as well as primary isolates from AIDS patients).[21] The cyclolignans 3′-demethoxyisoguaiacin and norisoguaiacin (Fig. 2B), also isolated from creosote bush, were significantly more active than all NDGA derivatives including Mal-4.[15] However, these compounds occur in much smaller amounts in the extract compared to NDGA and its analogs. Full synthetic methylation of NDGA yielded the tetramethylated derivative terameprocol (M4N; Fig. 3B), which has advanced to clinical trials as an anticancer agent.[22] M4N is an amorphous powder that naturally occurs in the plant, but in much lower concentration than NDGA and many NDGA derivatives.

The viral gene transcription machinery of many family viruses is made of complex structures containing a scaffold of multiple proteins and transcription factors interacting with each other and with the viral DNA to promote viral gene expression.[23] Specificity Protein One (Sp1) is a crucial transcriptional regulatory element controlling > 1000 genes and is found at one or several motifs in the promoters of several family viruses. HIV contains three Sp1 motifs, which directly and indirectly interact with the HIV Tat protein to

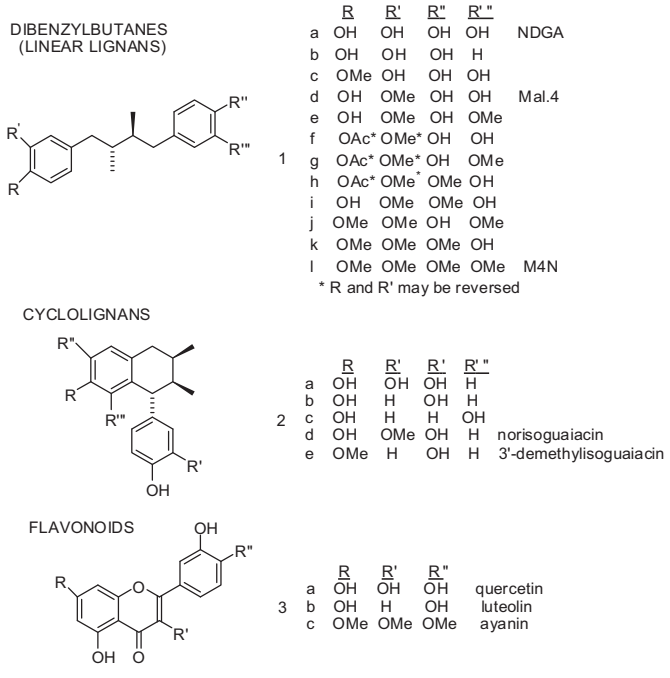


**Fig. 2.** Three classes of compounds isolated from the creosote bush and contained in the proprietary Maximum Extract 1 (Me-1) formulation: dibenzylbutanes (linear lignans), cyclolignans, and flavonoids. Within these three groups, the compounds vary mostly in the number and positions of hydroxyl and methoxyl groups. The extract also contains smaller amounts of saponins, sterols, tannins, and other compounds[9].

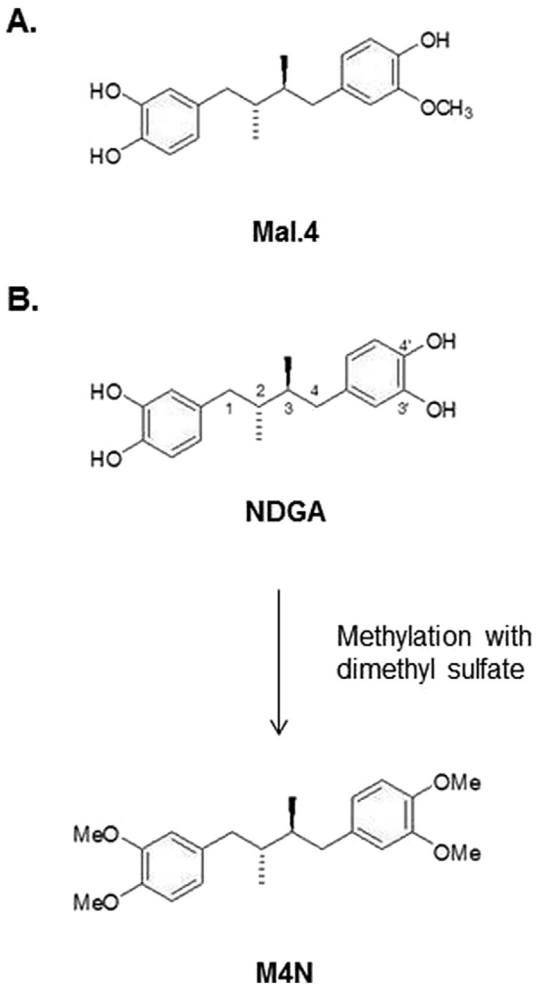


**Fig. 3.** Important methylated lignans from creosote bush of medical relevance: Antiviral 3′-O-methyl nordihydroguaiaretic acid (Mal-4) and anticancer tetra-O-methylnordihydroquaiaretic acid (M4N). Other lignans isolated from the creosote bush also possess antiviral and anticancer activities.

exponentially increase the virus gene transcription and replication. The creosote bush lignans have structural determinants that bind to DNA sequences rich in cytosine/guanine (C/G) boxes and Sp1 is likely to be a primary target for these compounds. The blockade of this sequence by the plant molecules (for instance M4N) prevents Sp1 factors from binding to their cognate site at HIV LTR promoter level. As a result, the virus replication is abrogated. Thus, these lignans are able specifically to inhibit HIV basal gene transcription as well as HIV Tat-regulated gene *trans*-activation.[21]

The sexually transmitted herpes simplex virus type 2 (HSV-2) affects ~ 500 million people worldwide. More than 80% of the world population is seropositive for HSV-1 according to WHO's reports.[24] Within 18 hours after infecting the cells, HSV makes three types of genes: the immediate early or α genes, then the intermediate or β genes and finally, the late or γ genes. For a successful infection, these genes must be made in that sequential order (α, then β, then γ). The α-gene ICP4 plays a pivotal role in this viral cascade and is an attractive therapeutic target, since without its expression, all early genes cannot be transcribed.[25]

Herpes virus has no effective vaccine but the infection can be managed by good symptomatic drug treatments, namely nucleoside/nucleotide analogues. More than a dozen drugs are currently on the market or in the pipeline of development for herpes infections.[26] They act as decoys that are inserted in the viral DNA

during synthesis and stop the elongation of the viral nucleic acid, thus temporarily suppressing the viral infection. Since these drugs target viral DNA synthesis and the ever-changing viral enzymes, they are prone to drug resistance in the long run.[27] Acyclovir (trade name Zovirax) has been intensively studied and is the gold standard for the treatment of HSV-1 and HSV-2.

None of the current anti-HSV drugs takes advantage of the presence of a highly conserved therapeutic target in the virus promoter. Indeed, the α-ICP4 promoter in all herpes simplex strains contains 8 Sp1 binding motifs, thus providing specific targets for the creosote bush lignans. Several studies have shown that NDGA derivatives suppress HSV infection *in vitro* and *in vivo*, with no indication of cytotoxicity or drug resistance. In fact, in a comparative study between acyclovir and M4N in acyclovir-resistant HSV strains, M4N was able to inhibit all acyclovir-resistant HSV strains to the $10^{th}$ passage (final endpoint) in Vero cells while inducing virtually no intrinsic cytotoxicity.[28] As a result, the spread of genital herpes infections, which is now a factor in the spread of HIV/AIDS,[29] and other sexually transmitted diseases including papilloma virus infection, could significantly be curbed worldwide.

Human papillomavirus (HPV) causes uncontrolled cell proliferation and uterine cervical intraepithelial neoplasia, which may become cancerous. According to a recent report of the WHO, each year 530,000 women develop cervical cancer and about 275,000 (52%) die from the disease. DNA sequencing of HPV genome shows that virus has more than 200 types. To our knowledge, no effective drug treatment of HPV has been reported yet. However, commercially available vaccines against the most virulent HPV types are currently in clinical use. Molecular studies show that all HPV types contain one Sp1 motif in their HPV-16 long control region promoter, which makes them a specific target for the creosote bush lignans. Indeed, Mal-4 has been shown to interfere with HPV type 16 early promoter p97 to suppress HPV replication via interaction with Sp1 transcription factor in p97 promoter.[30] These and other *in vitro* studies on the anti-HPV activities of the plant lignans are corroborated by conclusive preclinical and clinical studies of terameprocol on the cervix of women for potential use of the drug as a microbicide.[31,32]

In summary, herpes viruses have a wide range of drug treatments but no vaccine. Human papillomavirus has vaccines but no drug treatment. The creosote bush lignans indiscriminately suppress the infections of both viruses. Therefore, the plant lignans inhibit the replication of at least three major sexually transmitted viruses (HIV, HPV, and HSV) by blocking the invariable Sp1 gene transcription cofactor that they share in common. This unique feature makes these plant molecules potential agents in a useful vaginal microbicide to stop sexual exposure to these viruses. It is noteworthy that beside these three viruses, Sp1 motifs are found in the viral gene promoters of a number of other viruses including blindness-causing cytomegalovirus and the potential tumor-causing simian vacuolating virus 40. Other newly emerging viruses may be responsive to these lignans as well.

#### 6.2.2. Anticancer activities

Carcinogenesis is a multistage event affected by a variety of genetic and epigenetic factors and is typified by the outbreak of uncontrolled cell growth originated from different tissues. A universal goal for anticancer research lies in the development of a clinical treatment that must meet three criteria: (1) the drug must be highly effective in curtailing tumor growth; (2) it must be nontoxic to the host; and (3) it must be affordable for most patients. Drugs that inhibit targets that are unique to dividing cells, particularly cells in an uncontrolled division, are an ideal paradigm for chemotherapeutic agents. The greater the specificity to cells that are dividing in an uncontrolled manner, the lower the risks of attendant side effects.

In addition to its antiviral activities, M4N has proved to be an effective anticancer agent.[33] Structural binding studies using a water soluble M4N analog have demonstrated that it interacts with G and C residues within the Sp1 consensus binding site (unpublished nuclear magnetic resonance data) resulting in a widening of the major groove of the DNA double helix and inhibition of Sp1 binding as demonstrated by crystallographic studies.[34] Preclinical studies have demonstrated that M4N suppresses the growth of a variety of mouse and human tumor cells and human tumor explants in nude mice.[35] Cancer cell growth retardation and cytotoxicity were shown to be in part the result of terameprocol's ability to block transcription of the Sp1-dependent genes CDC2 and surviving, leading to cell cycle arrest and apoptotic death of the tumor cells.[36,37] M4N is currently in clinical trials and has been found safe for human use.[38]

Tumor cells often become resistant to chemotherapy that was initially effective. One mechanism of drug resistance that has been described is over-expression of the multiple drug resistance gene (*ABCB1*) encoding a transmembrane glycoprotein (P-glycoprotein). This protein is one of a family of proteins involved in plasma membrane permeability that function as efflux pumps for numerous lipophilic anticancer drugs.[39] The P-gp/MDR1 protein is over-expressed in drug resistant neoplastic cells and its gene promoter is regulated by the Sp1 transcription factor, which is an oncogenic factor.[40] Methylated NDGA derivatives have been shown to induce a reversal of multidrug resistance of breast cancer cells to doxorubin or paclitaxel, key chemiotherapeutic agents of the deadly disease.[33]

In summary, the molecular mechanisms underlying the anticancer activities of creosote bush lignans have been shown to involve a dozen cancer pathways, and this number is on the rise as research progresses. The specific inhibition of the nonmutable Sp1 oncogenic factor and all the mechanisms under its control including the drug resistance efflux pump, along with the relative non-toxicity of these plant lignans, represent key factors that make the creosote bush molecules of important clinical relevance for the treatment and prevention of current and future diseases.

#### 6.2.3. Activity against neurodegenerative disorders and senescence

The activities of the plant lignans against neurodegenerative disorders and aging symptoms are crucial since these disorders have taken a heavy toll on industrial nations with long living populations. The detailed studies will be published elsewhere, but here we summarize the recent data on these lignans.

As dreadful as cancer is, the disease is not always caused by genomic instability or family inheritance of abnormal genes, which contributes to < 10% of carcinogenesis. About 90% of all cancers result from risk factors, namely environmental factors and lifestyle.[41] These risk factors are: cigarette smoke, alcoholic beverages, environmental pollutants (pesticides, gasoline exhausts, industrial pollutants), sun UV sunlight, food (fried, grilled, smoked), red meat consumption, cancer-causing infectious agents (HIV, HPV, *Helicobacter pylori*, hepatitis B virus, hepatitis C virus, etc.), stress, obesity, hyperglycemia, lack of physical exercises, etc. The link between these risk factors and cancer promotion is the production of proinflammatory mediators such as cytokines, chemokines, reactive oxygen species, and inflammatory enzymes.[42] It is established that most of these risk factors are also etiological for neurodegenerative disorders and premature ageing of human brain cells. Reactive oxygen species are now directly implicated in a variety of neurological disorders including cerebral ischemia, Alzheimer's disease, acceleration of neuronal senescence, and loss of brain cells.[43]

The creosote bush lignans have been known for decades for their strong anti-inflammatory activities. They block the production of these proinflammatory mediators by inhibiting the enzymes responsible for the inflammatory cascades.[44] This partially

accounts for the activity of these plant molecules against asthma, type II diabetes,[45] rheumatoid arthritis, vascular diseases,[46] etc., and their remarkable neuroprotective effects were recently described in an excellent review.[14] Increasing data also support the beneficial effects of the plant lignans against Alzheimer's disease and retardation of senescence (aging symptoms), as Miller and his team[47,48] demonstrated on animal models of the disease.

All these multifaceted activities of the plant lignans justify the various medicinal applications of the creosote bush extract in the traditional sphere. It is our postulate that these plant lignans along with their flavonoid congeners (also active), could represent an important natural cocktail for the treatment of major human ailments such as viral infections, cancer, and neurodegenerative diseases, as the ethnobotanical data have shown for decades among indigenous populations.

## 7. A lignan-flavanoid combination cocktail for the treatment of cancers and viruses

Targeted therapy of cancer has proved beneficial and has saved countless lives, but the cure rate remains ~ 25%. This translates to a 75% failure rate. Because cancer cells are continually undergoing genetic changes, cancer eradication will require a multitargeted approach to inhibit all of the biological pathways involved. Similarly, it is now established that for an effective control of HIV/AIDS, a combination of antiretroviral drugs is required to suppress virus replication in patients. A combination of the creosote bush compounds differs from established drug regimens for cancer and virus infections, since the plant molecules are relatively safe, have synergistic effects, and circumvent the development of drug resistance. We show in this review that a combination of lignans and flavonoids of the creosote bush may represent a safe and effective asset from nature to combat these diseases.

The Maximum Extract 1 (Me-1) formulation (Fig. 4C) is a new strategy based on principles of traditional medicine and modern targeted therapy. Plant lignans and flavonoids suppress a significant number of cancer pathways. In addition, lignans inhibit the multidrug resistant efflux pump, and thus potentiate most anticancer agents as well as other phytochemicals. The combined action of the lignans and flavonoids appears to be synergistic, eliciting much greater activity than when one class of compounds is used alone. The clinical use of creosote bush extract is not new. One of the most

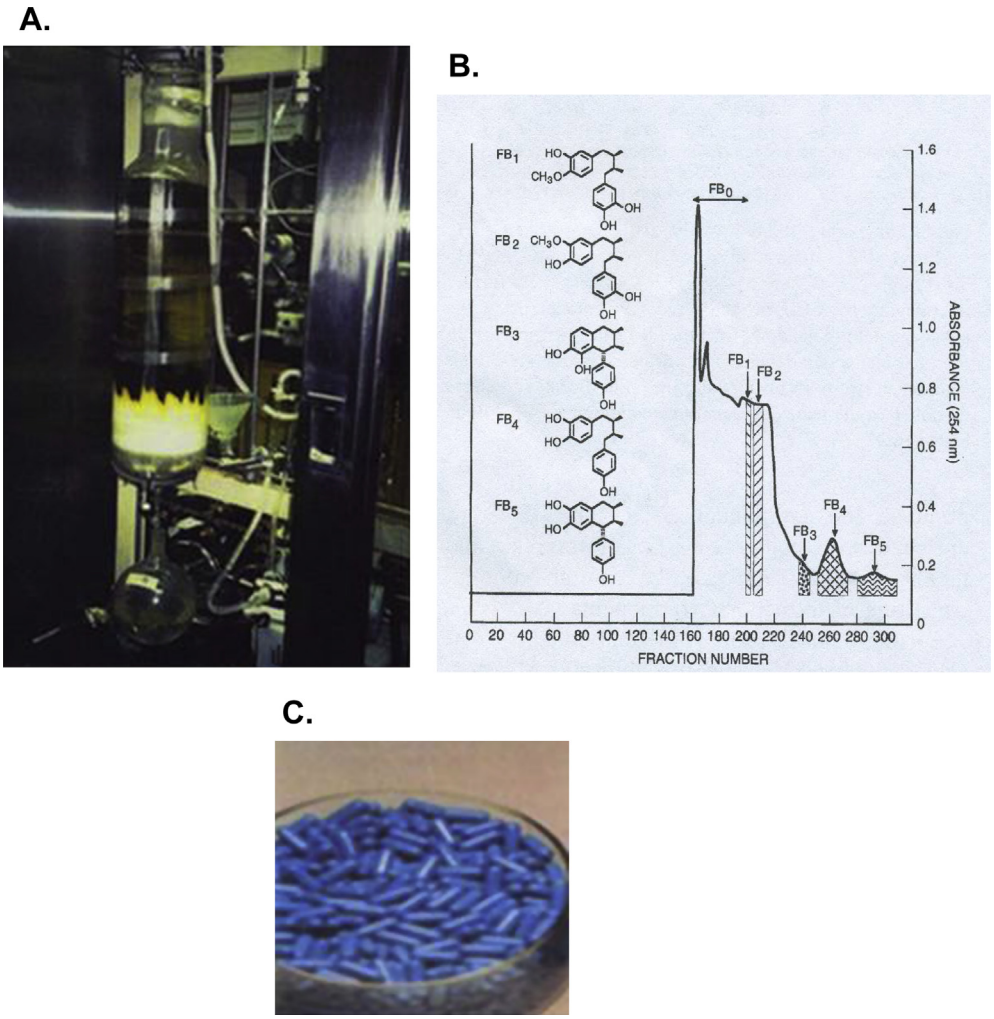


**Fig. 4.** (A) Flash chromatography of creosote bush total extract to remove the bulk of volatile compounds (dark band eluting with solvent front), which is mainly responsible for the plant toxicity (personal communication). (B) Countercurrent chromatographic (CCC) purification of active components of the detoxified extract. A chromatographic fingerprint of the semipurified extract was first established. Active peaks were pooled into two majors fractions (Gr and Lo) based on their TLC patterns, and further resolved by CCC. The isolation studies are published elsewhere.[15] All compounds were characterized by nuclear magnetic resonance and mass spectrometry. As shown on Fig. 2, Me-1 formulation contains cyclolignans, several flavonoids and all linear lignans without NDGA, the cyclolignans, and several flavonoids, as shown on Fig. 2 (C) Blue capsules. Me-1 extract was formulated into blue capsules by a proprietary pharmaceutical capsule formulation after animal studies for safety (Table 1). This final product encompasses all compounds of Fig. 2, including inactive ingredients of capsule formulation. The blue capsules demonstrated greater efficacy than any individual lignan when tested on humans as a dietary supplement.

popular medicinal formulations of the creosote bush are chaparral in tea and in tincture. Chaparral has been evaluated in clinical studies for intrinsic toxicity[49] as well as for anticancer activities.[50] The conclusion of these studies emphasized a potential liver toxicity of chaparral when used in a high dose,[51] which, according to our findings, is due to a significant presence of essential oils.

Our studies also showed that chaparral tea and tincture contain only 10% and 25%, respectively of the active methylated lignans in the total extract. To develop a cocktail of the beneficial natural products of the creosote bush, the extract of the plant leaves was first detoxified by flash silica gel column chromatography to remove the essential oils (Fig. 4A). The semipurified extract (called Me-1) was analyzed using countercurrent chromatography (CCC), and a reference fingerprint chromatogram of the extract was established. Thin-layer chromatography analysis enabled the identification of all the peaks of anti-HIV active lignans in two pools (Gr and Lo), which were individually resolved based on the separation conditions of our previous study.[15] (An example of the purification of Lo fraction is illustrated in Fig. 4B.) The cocktail of Me-1 was generated by gathering all the compounds resulting from the separation studies, and after their chemical characterization by nuclear magnetic resonance and mass spectrometry. As shown in Fig. 2, Me-1 consists mostly of three classes of compounds: (1) the 11 dibenzylbutane lignans (without NDGA but including M4N and Mal-4); (2) the five very active cyclolignans; and (3) several flavonoids including quercetin, luteolin, and methylated flavonoids such as ayanin, devoid of anti-HIV activity, were included into the formulation for their anticancer effects.

Interestingly, most compounds in Me-1 formulation have various degrees of methylation. Methylated molecules are more lipophilic, and therefore can more readily permeate lipid bilayer cell membranes and virus envelope to disrupt the targeted mechanisms inside. All these compounds should work synergistically as they compete for various targets inside the cells and viruses. It is our view that for a robust and effective treatment of complex diseases such as cancer, a combination of small organic molecules is required, and these molecules must fulfill three major criteria: (1) they must be relatively safe, sparing normal cells and reversing multidrug resistance phenotype of cancer cells; (2) they must have multi-ligand capability, i.e., each molecule in the cocktail must act on multiple biomolecular targets; and, finally, (3) they must be capable of reversing tumorigenesis by inhibiting oncogenes or restoring the tumor suppressor genes such as the p53 family of tumor suppressor proteins. It is our hypothesis that Me-1 formulation meets all these requirements, and thus should be a complement to current chemotherapy, or a valid alternative to cancer treatment.

As for safety, Me-1 was formulated into oral capsules after toxicity testing in animals showed that the product was devoid of acute toxicity (Table 1). The United States Food and Drug Administration, which audited the studies, gave their stamp of approval for safety of the formulation. The bioassays showed that the cocktail was significantly more active than each major NDGA derivative (M4N, Mal-4), when tested against various cancer cell lines (data not shown). This activity was even greater by several hundred-fold in *in vivo* studies. These results were corroborated by other investigators who demonstrated that organic extracts of the creosote bush exhibited greater activity than individual compounds including NDGA and the cancer drug paclitaxel when tested against breast cancer cell lines (MCF7).[52] These preliminary results lend validity to our view that the Me-1 strategy may lead to a safer and more effective anticancer and antiviral cocktail. This formulation may be a bridge between Eastern herbal-based medicines and Western single synthetic molecule-based chemotherapy. Since Western medicine has begun to embrace the use of drug combinations, we postulate that our Me-1 formulation may represent the trend of future medicines for complex diseases.

**Table 1**
Toxicity testing of the Maximum Extract 1 (Me-1) formulation on animals. The purified Me-1 was formulated to be included in the animal feed and submitted to a month-long acute toxicity studies on rats (about 300 g females). The animals were divided into four groups of four animals/cage. The rats in the first cage received no drug in their feed (placebo). Rats in the second cage were given a dose of Me-1 five times (× 5) the equivalent of the human dose (200 mg for a 70 kg person). Rats in the remaining two cages received 15 times (× 15) and 50 times (× 50) the equivalent of the human dose, respectively. All the animals were fed for 28 days with Me-1 product under identical conditions as the placebo group. At the end of the study, the animals were sacrificed and all blood parameters, liver enzymes, and histology of the major organs were analyzed by state-of-the-art methods. There was no statistically significant difference between the drug-treated groups and the placebo, neither was any significant difference found among the drug-treated groups. Me-1 was then formulated into blue capsules using a proprietary pharmaceutical method of capsule formulation.

| Drug | Dose (in feed daily for 1 mo) | Toxicity (compared to placebo) |
|---|---|---|
| Placebo (No Me-1) | 0 mg | |
| Me-1 (× 5) | 4.3 mg | No significant difference |
| Me-1 (× 15) | 12.9 mg | No significant difference |
| Me-1 (× 50) | 42.9 | No significant difference |

## 8. Conclusion and perspectives

The accumulated scientific data on creosote bush lignans lend credentials to the multi-faceted uses of the plant in the traditional sphere. These plant products are especially effective against viral diseases and cancer. For millennia, the creosote bush has served as an important source for folk medicine to several indigenous populations of South and North America. Only in the past few decades has the ethnopharmacological history of the plant generated considerable interest for investigations of the active principles responsible for the multipurpose uses of the plant in traditional medicine.

Viruses such as HIV, HSV, influenza viruses, and cold viruses infect and kill millions of people every year. Severe acute respiratory syndrome, avian flu viruses, Middle East respiratory syndrome corona virus, Ebola virus, and many others are still without treatments and some may become global pandemics if not urgently addressed. The few vaccines available against some of these viruses need to be updated every year, and never reach the majority of people in developing nations. Antibiotics are designed to reach and stop the replication of bacteria without crippling the vital cellular genetic machinery. By contrast, most viruses have the ability to hijack the cell genetic machinery inside the nucleus in order to replicate. This explains why antibiotics are often ineffective against many viral infections such as the flu, cold virus, and many others. The creosote bush lignans possess the ability to act at the cellular level and permeate the nucleus and the virus envelope to block the viral gene machinery.[21] They are capable of suppressing viruses that contain Sp1 or are rich in C/G boxes in their gene transcriptional machinery such as HSV, varicella zoster virus, blindness-causing cytomegalovirus, HIV, and HPV. Since Sp1 and C/G motifs are common features in many virus families and are highly conserved (nonmutable), this class of plant lignans could represent a breakthrough for viral treatment as penicillin became for bacteria since World War II.

In this review, we presented the most recent findings on the active ingredients of the creosote bush, especially the lignans. We showed that the use of these compounds to treat and prevent modern diseases, some of which have no cure, demonstrates the relevance of traditional medicine as a pathfinder to the search for

molecules with new applications. Since the current diseases are complex and involve multiple pathways, a cocktail of safe but effective molecules is the best strategy to defeat these diseases.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

## Acknowledgments

The authors would like to thank David E. Mold, MD, PhD and Elham Mohammadi, PhD for help in editing the manuscript.

## References

1. Van der Crabben J. Medicine. In: *Ancient History Encyclopedia*; available at: www.ancient.eu.com/medicine Accessed 05.08.11.
2. Mahady GB. Global harmonization of herbal health claims. *J Nutr*. 2001;131:1120S–1123S.
3. Xu H, Lv M, Tian X. A review on hemisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: 2003–2007. *Curr Med Chem*. 2009;16:327–349.
4. Ionkova I. Anticancer lignans: from discovery to biotechnology. *Mini Rev Med Chem*. 2011;11:843–856.
5. Panossian A, Wikman G. Pharmacology of *Schisandra chinensis* Bail.: an overview of Russian research and uses in medicine. *J Ethnopharmacol*. 2008;118:183–212.
6. Arteaga S, Andrade-Cetto A, Cárdenas R. *Larrea tridentata* (Creosote bush), an abundant plant of Mexican and US-American deserts and its metabolite nordihydroguaiaretic acid. *J Ethnopharmacol*. 2005;98:231–239.
7. Lia VV, Confalonieri VA, Comas CI, Hunziker JH. Molecular phylogeny of *Larrea* and its allies (Zygophyllaceae): reticulate evolution and the probable time of creosote bush arrival to North America. *Mol Phylogenet Evol*. 2001;21:309–320.
8. Laport RG, Minckley RL, Ramsey J. Phylogeny and Cytogeography of the North American Creosote Bush (*Larrea tridentata*, Zygophyllaceae). *Systematic Botany*. 2012;37:153–164.
9. Mabry TJ, Hunziker JH, DiFeo Jr DR. *Creosote Bush: Biology and Chemistry of Larrea in the New World Deserts*. Stroudsburg, PA: Dowden: Hutchinson and Ross, Inc.; 1977.
10. Konno C, Lu ZZ, Xue HZ, et al. Furanoid lignans from *Larrea tridentata*. *J Nat Prod*. 1990;53:396–406.
11. Haworth RD. Natural resins. *Ann Rep Prog Chem*. 1936;33:267–278.
12. Zhang J, Chen J, Liang Z, Zhao C. New lignans and their biological activities. *Chem Biodivers*. 2014;11:1–54.
13. Macrae DW, Towers NGH. Biological activities of lignans. *Phytochemistry*. 1984;23:1207–1220.
14. Lü JM, Nurko J, Weakley SM, et al. Molecular mechanisms and clinical applications of nordihydroguaiaretic acid (NDGA) and its derivatives: an update. *Med Sci Monit*. 2010;16:RA93–RA100.
15. Gnabre JN, Ito Y, Ma Y, Huang RC. Isolation of anti-HIV-1 lignans from *Larrea tridentata* by counter-current chromatography. *J Chromatogr A*. 1996;719:353–364.
16. Sok DE, Cui HS, Kim MR. Isolation and bioactivities of furfuran type lignan compounds from edible plants. *Recent Pat Food Nutr Agric*. 2009;1:87–95.
17. Gnabre J, Unlu I, Chang T-C, et al. Isolation of lignans from *Schisandra chinensis* with anti-proliferative activity in human colorectal carcinoma: structure–activity relationships. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010;878:2693–2700.
18. Lee YJ, Lee YM, Lee CK, Jung JK, Han SB, Hong JT. Therapeutic applications of compounds in the Magnolia family. *Pharmacol Ther*. 2011;130:157–176.
19. Lambert JD, Dorr RT, Timmermann BN. Nordihydroguaiaretic acid, a review of its numerous and varied biological activities. *Pharm Biol*. 2004;42:149–158.
20. Lambert JD, Zhao D, Meyers RO, Kuester RK, Timmermann BN, Dorr RT. Nordihydroguaiaretic acid: hepatotoxicity and detoxification in the mouse. *Toxicon*. 2002;40:1701–1708.
21. Gnabre JN, Brady JN, Clanton DJ, et al. Inhibition of human immunodeficiency virus type 1 transcription and replication by DNA sequence-selective plant lignans. *Proc Natl Acad Sci USA*. 1995;92:11239–11243.
22. Lopez RA, Goodman AB, Rhodes M, Blomberg JA, Heller J. The anticancer activity of the transcription inhibitor terameprocol (meso-tetra-O-methyl nordihydroguaiaretic acid) formulated for systemic administration. *Anticancer Drugs*. 2007;18:933–939.
23. Ruelas DS, Greene WC. An integrated overview of HIV-1 latency. *Cell*. 2013;155:519–529.
24. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and1: a global review. *J Infect Dis*. 2002;186(suppl 1):S3–S28.
25. Jenkins FJ, Turner SL. Herpes simplex virus: a tool for neuroscientists. *Front Biosci*. 1996;1:d241–d247.
26. Dalfour Jr HH. Antiviral drugs. *N Engl J Med*. 1999;340:1255–1268.
27. Piret J, Guy Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrob Agents Chemother*. 2011;55:459–572.
28. Chen H, Teng L, Li JN, et al. Antiviral activities of methylated nordihydroguaiaretic acids. 2. Targeting herpes simplex virus replication by the mutation insensitive transcription inhibitor tetra-O-methyl-NDGA. *J Med Chem*. 1998;41:3001–3007.
29. Shin H, Iwasaki A. Generating protective immunity against genital herpes. *Trends Immunol*. 2013;34:487–494.
30. Craigo J, Callahan M, Huang RC, DeLucia AL. Inhibition of human papillomavirus type 16 gene expression by nordihydroguaiaretic acid plant lignan derivatives. *Antivir Res*. 2000;47:19–28.
31. Khanna N, Dalby R, Tan M, Arnold S, Stern J, Frazer N. Phase I/II clinical safety studies of terameprocol vaginal ointment. *Gynecol Oncol*. 2007;107:554–562.
32. Khanna N, Dalby R, Connor A, Church A, Stern J, Frazer N. Phase I clinical trial of repeat dose terameprocol vaginal ointment in healthy female volunteers. *Sex Transm Dis*. 2008;35:577–582.
33. Chang CC, Liang YC, Klutz A, et al. Reversal of multidrug resistance by two nordihydroguaiaretic acid derivatives, M4N and maltose-M3N, and their use in combination with doxorubicin or paclitaxel. *Cancer Chemother Pharmacol*. 2006;58:640–653.
34. Dohm JA, Hsu MH, Hwu JR, et al. Influence of ions, hydration, and the transcriptional inhibitor P4N on the conformations of the Sp1 binding site. *J Mol Biol*. 2005;349:731–744.
35. Park R, Chang CC, Liang YC, et al. Systemic treatment with Tetra-*O*-methyl nordihydroguaiaretic acid suppresses the growth of human xenograft tumors. *Clin Cancer Res*. 2005;11:4601–4609.
36. Chang CC, Heller JD, Kuo J, Huang RC. Tetra-O-methyl nordihydroguaiaretic acid induces growth arrest and cellular apoptosis by inhibiting Cdc2 and survivin expression. *Proc Natl Acad Sci USA*. 2004;101:13239–13244.
37. Huang RC, Chang CC, Mold D. Survivin-dependent and -independent pathways and the induction of cancer cell death by tetra-O-methyl nordihydroguaiaretic acid. *Semin Oncol*. 2006;33:479–485.
38. Grossman SA, Ye X, Peereboom D, et al. Phase I study of terameprocol in patients with recurrent high-grade glioma. *Neuro Oncol*. 2012;14:511–517.
39. Breier A, Gibalova L, Seres M, Barancik M, Sulova Z. New insight into P-glycoprotein as a drug target. *Anticancer Agents Med Chem*. 2013;13:159–170.
40. Bailey-Dell KJ, Hassel B, Doyle LA, Ross DD. Promoter characterization and genomic organization of the human breast cancer resistance protein (ATP-binding cassette transporter G2) gene. *Biochim Biophys Acta*. 2001;1520:234–241.
41. Anand P, Kunnumakkara AB, Sundaram C, et al. Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res*. 2008;25:2097–2116.
42. Steinhilber D, Fischer AS, Metzner J, et al. 5-lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis. *Front Pharmacol*. 2010;1:143.
43. Noor R, Mittal S, Iqbal J. Superoxide dismutase: applications and relevance to human diseases. *Med Sci Monit*. 2002;8:RA210–RA215.
44. Eads D, Hansen R, Oyegunwa A, et al. Terameprocol, a methylated derivative of nordihydroguaiaretic acid, inhibits production of prostaglandins and several key inflammatory cytokines and chemokines. *J Inflamm (Lond)*. 2009;6:2.
45. Luo J, Chuang T, Cheung J, et al. Masoprocol (nordihydroguaiaretic acid): a new antihyperglycemic agent isolated from the creosote bush (*Larrea tridentata*). *Eur J Pharmacol*. 1998;346:77–79.
46. Pitocco D, Tesauro M, Alessandro R, Ghirlanda G, Cardillo C. Oxidative stress in diabetes: implications for vascular and other complications. *Int J Mol Sci*. 2013;14:21525–21550.
47. Miller RA, Harrison DE, Astle CM, et al. An aging interventions testing program: study design and interim report. *Aging Cell*. 2007;6:565–575. Erratum in: Aging Cell. 2008;7:445.
48. Strong R, Miller RA, Astle CM, et al. Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. *Aging Cell*. 2008;7:641–650.
49. Heron S, Yarnell E. The safety of low-dose *Larrea tridentata* (DC) Coville (creosote bush or chaparral): a retrospective clinical study. *J Altern Complement Med*. 2001;7:175–185.
50. Smart CR, Hogle HH, Vogel H, Broom AD, Bartholomey D. Clinical experience with nordihydroguaiaretic acid—'chaparrel tea' in the treatment of cancer. *Rocky Mt Med J*. 1970;67:39–43.
51. Sheikh NM, Philen RM, Love LA. Chaparral-associated hepatotoxicity. *Arch Intern Med*. 1997;157:913–919.
52. Bongiovanni G, Cantero J, Eynard A, Goleniowski M. Organic extracts of *Larrea divaricata* Cav. induced apoptosis on tumoral MCF7 cells with an higher cytotoxicity than nordihydroguaiaretic acid or paclitaxel. *J Exp Ther Oncol*. 2008;7:1–7.