

**(12) STANDARD PATENT**  
**(19) AUSTRALIAN PATENT OFFICE**

(11) Application No. **AU 2011323155 B2**

(54) Title  
**Combination therapy for prostate cancer using botanical compositions and bicalutamide**

(51) International Patent Classification(s)  
**A01N 65/00** (2006.01)

(21) Application No: **2011323155** (22) Date of Filing: **2011.11.04**

(87) WIPO No: **WO12/061790**

(30) Priority Data

(31) Number	(32) Date	(33) Country
<b>61/410,327</b>	<b>2010.11.04</b>	<b>US</b>

(43) Publication Date: **2012.05.10**

(44) Accepted Journal Date: **2016.05.12**

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(56) Related Art  
**US 2009/0130118 A1**  
**US 2005/0208070 A1**

(19) World Intellectual Property Organization  
International Bureau

(43) International Publication Date  
10 May 2012 (10.05.2012)



(10) International Publication Number  
**WO 2012/061790 A1**

- (51) **International Patent Classification:**  
A01N 65/00 (2009.01)
- (21) **International Application Number:**  
PCT/US2011/059471
- (22) **International Filing Date:**  
4 November 2011 (04.11.2011)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
61/410,327 4 November 2010 (04.11.2010) US
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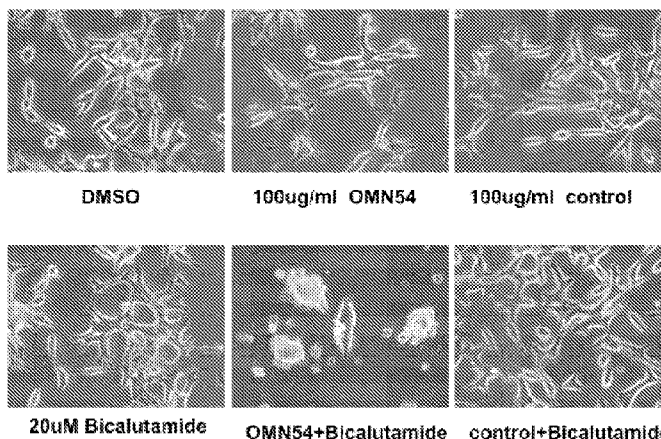
(81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ,

[Continued on next page]

(54) **Title:** COMBINATION THERAPY FOR PROSTATE CANCER USING BOTANICAL COMPOSITIONS AND BICALUTAMIDE

FIGURE 5



(57) **Abstract:** Botanical compositions comprising non-alcoholic organic extracts of Ganoderma lucidum, Salvia miltiorrhiza, and Scutellaria barbata for use in conjunction with bicalutamide therapy for cancer therapy, are provided. Methods for treatment or therapy of prostate cancer in a human is provided, the method comprising: administering an effective amount of a botanical composition that is effective for reducing androgen receptor protein expression; and administering concurrently an effective amount of a compound having anti-androgen activity, wherein the concurrent administration of the compound and the botanical composition achieves a therapeutic effect that is more effective than either agent alone.

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DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT,  
LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS,  
SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

## COMBINATION THERAPY FOR PROSTATE CANCER USING BOTANICAL COMPOSITIONS AND BICALUTAMIDE

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### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority of U.S. Provisional Patent Application Serial No. 61/410,327, filed November 4, 2010 and titled "BOTANICAL COMPOSITIONS AND BICALUTAMIDE FOR PROSTATE CANCER," the contents of which are incorporated herein in their entirety by reference.

### TECHNICAL FIELD OF THE INVENTION

[0002] This invention relates generally to the field of compositions for treatment of cancer. More specifically, the invention provides multifunctional, multitargeted compositions of botanical extracts in combination with bicalutamide for the prevention and therapy of cancer, and specifically prostate cancer.

### BACKGROUND OF THE INVENTION

[0003] Prostate cancer is cancer that forms in tissues of the prostate (a gland in the male reproductive system found below the bladder and in front of the rectum). Prostate cancer usually occurs in older men. According to the National Cancer Institute, it is estimated that 217,730 new cases and 32,050 deaths from prostate cancer in the United States in 2010.

[0004] Most prostate cancers are slow growing; however, there are cases of aggressive prostate cancers. The cancer cells may metastasize (spread) from the prostate to other parts of the body, particularly the bones and lymph nodes. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, or erectile dysfunction. Other symptoms can potentially develop during later stages of the disease.

[0005] Treatment options for prostate cancer with intent to cure are primarily surgery, radiation therapy, and proton therapy. Other treatments, such as hormonal therapy, chemotherapy, cryosurgery, and high intensity focused ultrasound (HIFU) also exist, depending on the clinical scenario and desired outcome.

[0006] The age and underlying health of the man, the extent of metastasis, appearance under the microscope, and response of the cancer to initial treatment are important in determining the

outcome of the disease. The decision whether or not to treat localized prostate cancer (a tumor that is contained within the prostate) with curative intent is a patient trade-off between the expected beneficial and harmful effects in terms of patient survival and quality of life

**[0007]** Bicalutamide (marketed as Casodex<sup>®</sup>, Cosudex<sup>®</sup>, Calutide<sup>®</sup>, Kalumid<sup>®</sup>) is an oral non-steroidal anti-androgen used in the treatment of prostate cancer (Schellhammer PF, Davis JW *Clin Prostate Cancer*. 2004 Mar;2(4):213-219) and hirsutism. (Müderris II, *et al.*, *Gynecol. Endocrinol.* **16** (1): 63–66 (2002)). It was first launched in 1995 as a combination treatment (with surgical or medical castration) for advanced prostate cancer and subsequently launched as monotherapy for the treatment of earlier stages of the disease. Bicalutamide is in a class of medications called nonsteroidal antiandrogens. It works by blocking the effect of androgen (a male hormone), to stop the growth and spread of cancer cells.

**[0008]** Bicalutamide is marketed by AstraZeneca with the brand names Casodex<sup>®</sup> and Cosudex<sup>®</sup>. It is recommended in combination with a luteinizing hormone-releasing hormone analog or surgical castration. Bicalutamide is used with another medication (luteinizing hormone-releasing hormone [LHRH]; such as leuprolide or goserelin) to treat stage D2 metastatic prostate cancer (cancer that started in the prostate and has spread to other parts of the body), or as a monotherapy. (Schellhammer PF, *et al.*, *Urology* **50** (3): 330–336 (1997)). It has also been used in clinical trials for ovarian cancer. (Levine D, *et al.*, *Cancer* **110** (11): 2448–2456 (2007)) It has also been used in combination with castration. (Klotz L, *et al.*, *Clin Prostate Cancer* **3** (4): 215–219 (2005)).

**[0009]** Bicalutamide along with the luteinizing hormone-releasing hormone may help stop the growth and spread of cancer cells but does not cure prostate cancer. Most advanced prostate cancer patients eventually become resistant to antiandrogen including bicalutamide therapy.

**[0010]** There is a need for enhanced prostate cancer therapy regimens that increase effectiveness of the therapy while reducing side effects and toxicity resulting from the chemotherapeutic treatment.

**[0011]** Compositions of botanicals comprising therapeutically effective amounts of two or more of an extract of *Ganoderma lucidum*, an extract of *Salvia miltiorrhiza* and an extract of *Scutellaria barbata* for prevention and therapy of cancer have been reported by Dao *et al.* (US Pat. App. Pub. No. 20050208070).

## SUMMARY OF THE INVENTION

[0012] The present invention relates to combinations of bicalutamide and compositions botanical extracts for treatment and therapy of prostate cancer.

[0013] The compositions of botanical extracts can be used to reduce or alleviate the side effects when used with standard non-botanical chemotherapies. Side effects are reduced by inhibiting inflammatory responses, modulating immune responses, reducing oxidative stress, modulating immune responses, inhibiting viral and microbial infections, modulating cell proliferative responses or other biological responses. The compositions of the invention may also alleviate side effects of standard therapeutic agents by balancing general biological responses against perturbations in specific biological pathways due to treatment with the therapeutic agent.

[0014] In a preferred embodiment, the composition comprises combinations of two or more extracts of *Ganoderma lucidum*, *Scutellaria barbata*, *Scutellaria baicalensis*, *Salvia miltiorrhiza*, and optionally, *Hippophae rhamnoides* (sea buckthorn).

[0015] In one embodiment, this method comprises treatment or therapy of prostate cancer in a human is provided, the method comprising: administering an effective amount of a botanical composition that is effective for reducing androgen receptor protein expression; and administering concurrently an effective amount of a compound having anti-androgen activity, wherein the concurrent administration of the compound and the botanical composition achieves more effective therapy than either agent alone. Botanical compositions comprising non-alcoholic organic extracts of *Ganoderma lucidum*, *Salvia miltiorrhiza*, and *Scutellaria barbata* in conjunction with bicalutamide therapy are used.

[0016] The present invention and other objects, features, and advantages of the present invention will become further apparent in the following Detailed Description of the Invention and the accompanying Figures and embodiments.

## BRIEF DESCRIPTION OF THE FIGURES

[0017] Figure 1 shows the effect of the combination of *Ganoderma lucidum*, *Scutellaria barbata*, and *Salvia miltiorrhiza* (OMN54) and bicalutamide treatment on C4-2 cells.

[0018] Figure 2 shows effect of OMN54 and bicalutamide treatment on AR expression in C4-2 cells by Western blot analysis.

[0019] Figure 3 shows the effect of combination of OMN54 and bicalutamide on clonogenic ability in C4-2 cells.

[0020] Figure 4 shows effect of combination of OMN54 and bicalutamide on clonogenic ability in C4-2 cells.

[0021] Figure 5 shows the effect of combination of OMN54 and bicalutamide on LNCaP cell growth. LNCaP cells were treated with OMN54 and bicalutamide either alone or combination for 48 hr.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

[0021a] Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

[0022] The term “plant” as used herein refers to seeds, leaves, stems, flowers, roots, berries, bark, or any other plant parts that are useful for the purposes described. For certain uses, it is preferred that the underground portion of the plant, such as the root and rhizoma, be utilized. The leaves, stems, seeds, flowers, berries, bark, or other plant parts, also have medicinal effects and can be used for preparing tea and other beverages, cream, and in food preparation.

[0023] The term “treatment” or “treating” or “therapy” as used herein, for purposes of the specification and claims, includes preventing, inhibiting, curing, or alleviating.

[0024] By the term “administering,” it is meant that the compositions are delivered to the host in such a manner that it can achieve the desired purpose. As mentioned the compositions can be administered by an effective route, such as orally, topically, rectally, etc.

[0025] “Synergism” may be measured by combination index (CI). The combination index method was described by Chou and Talalay. (Chou, T.-C. The median-effect principle and the combination index for quantitation of synergism and antagonism, p. 61-102. *In* T.-C. Chou and D. C. Rideout (ed.), *Synergism and antagonism in chemotherapy*. Academic Press, San Diego, Calif. (1991); Chou, T.-C, and P. Talalay. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs on enzyme inhibitors. *Adv. Enzyme Regul.* 22:27-55 (1984)). A CI value of 0.90 or less is considered synergistic, with values of 0.85 being moderately

synergistic and values below 0.75 being significantly synergistic. CI values of 0.90 to 1.10 are considered to be merely additive and higher values are antagonistic.

**Table 1. Synergism/antagonism as a function of CI values**

<b>Combination Index (CI) Value</b>	<b>Interpretation</b>
>10	Very strong antagonism
3.3 – 10	Strong antagonism
1.45 – 3.3	Antagonism
1.2 – 1.45	Moderate antagonism



1.1 – 1.2	Slight antagonism
0.9 – 1.1	Additive
0.85 – 0.9	Slight synergism
0.7 – 0.85	Moderate synergism
0.3 – 0.7	Synergism
0.1 – 0.3	Strong synergism
< 0.1	Very strong synergism

[0026] It is noted that determination of synergy may be affected by biological variability, dosage, experimental conditions (temperature, pH, oxygen tension, etc.), treatment schedule and combination ratio.

[0027] A botanical composition drug include: bio-availability and minimal toxicity. Preferably the drug can be administered orally. The botanical composition provides a combination of multiple therapeutic functions to act simultaneously and synergistically on multiple biological targets. The botanical composition comprises low doses of individual therapeutic ingredients to minimize disruption of physiological homeostasis and development of drug resistance. Typically, a history of therapeutic efficacy and safety is considered in selecting nature-derived active ingredients.

**Cancer:**

[0028] Several cellular pathways have been implicated in cancer. Apoptotic pathway, mitogen-activated protein kinase (MAPK) Signaling Pathway, cell signaling via the phosphoinositide 3-kinase (PI3K) pathway, Signal transducers and activators of transcription (STAT) signaling pathway, p53 signaling pathway, *Wnt* signaling pathway, cyclooxygenase (COX) enzymes have all been known to contribute to several steps involved in tumor formation, such as neoplastic transformation, metastasis, and angiogenesis. Also, growth factors, oncogenes such as Ras mutations, tumor suppressor genes, androgen and estrogen receptors, co-activators & repressors, and numerous others pathways have been shown to affect tumor formation and carcinogenesis.

[0029] A botanical drug suitable for cancer would include functions to affect one or more of these pathways as well as functions generally related to alleviation of cancer conditions such as anti-inflammation, immune system modulation, anti-angiogenic, anti-metastatic, and the like.

[0030] A botanical drug suitable for a particular type of cancer will possess functionalities that also are directed to pathways unique to a type of cancer. For example, botanical drug suitable for the treatment of prostate cancer may include functionalities related to androgen receptors.

Preferably, the botanical drug is administered in combination with standard chemotherapy regimens to achi

**[0031]** Prostate cancer is a complex disease. A number of biological pathways have been implicated in prostate cancer development: growth factor activity, cell death (apoptosis), oncogenesis, tumor suppression, cell cycle modulation, cell surface modulation, androgen receptors, co-activators & repressors. Several conditions are associated with prostate disease: Benign prostate hyperplasia (BPH), prostatitis, prostatic intraepithelial neoplasia (PIN).

**[0032]** Benign prostatic hyperplasia (BPH) refers to the increase in size of the prostate in middle-aged and elderly men. BPH is characterized by hyperplasia of prostatic stromal and epithelial cells, resulting in the formation of large, fairly discrete nodules in the periurethral region of the prostate. Although prostate specific antigen levels may be elevated in these patients, because of increased organ volume and inflammation due to urinary tract infections, BPH is not considered to be a premalignant lesion.

**[0033]** Alpha blockers ( $\alpha$ 1-adrenergic receptor antagonists) provide symptomatic relief of BPH symptoms. When 5 $\alpha$ -reductase inhibitors are used together with alpha blockers a reduction of BPH progression to acute urinary retention and surgery has been noted in patients with enlarged prostates. (Kaplan SA, McConnell JD, Roehrborn CG, et al (2006). Combination therapy with doxazosin and finasteride for benign prostatic hyperplasia in patients with lower urinary tract symptoms and a baseline total prostate volume of 25 ml or greater. J Urol 175(1): 217-20.)

**[0034]** Several botanicals show effectiveness in treating BPH and can be used as components of a botanical formulation. *Serenoa repens* (saw palmetto) fruit extracts are alleviating mild-to-moderate BPH symptoms with comparable efficacy to finasteride. (Wilt TJ, Ishani A, MacDonald R, (2002). *Serenoa repens* for benign prostatic hyperplasia. Cochrane Database Syst Rev 2002 (3), CD001423) Other botanicals effective in treating BPH include beta-sitosterol from *Hypoxis rooperi* (African star grass), pygeum (extracted from the bark of *Prunus africana*), *Cucurbita pepo* (pumpkin) seed and *Urtica dioica* (stinging nettle) root. (Wilt TJ, Ishani A, Rutks I, MacDonald R (2000) Phytotherapy for benign prostatic hyperplasia Public Health Nutr 3(4A):459-72). One double-blind trial has also supported the efficacy of rye flower pollen. (Buck AC, Cox R, Rees RWM, et al. (1990) Treatment of outflow tract obstruction due to benign prostatic hyperplasia with the pollen extract, Cernilton. A double-blind placebo-controlled study Br. J. Urol. 66:398-404).

**[0035]** Prostate cancer is classified as an adenocarcinoma, or glandular cancer, that begins when normal semen-secreting prostate gland cells mutate into cancer cells. Initially, small clumps of

cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma in situ or prostatic intraepithelial neoplasia (PIN). Although there is no clear evidence that PIN is a cancer precursor, it is closely associated with cancer.

[0036] Prostate specific antigen (PSA) is a 34 kD glycoprotein manufactured almost exclusively by the prostate gland. Also known as kallikrein III, PSA is a serine protease. (Lilja H. (Nov 2003). "Biology of Prostate-Specific Antigen". *Urology* 62 ((5 Suppl 1)): 27-33).

[0037] PSA is often elevated in the presence of prostate cancer and in other non-malignant prostate disorders such as BPH. A blood test to measure PSA is the most effective test currently available for the early detection of prostate cancer. Higher than normal levels of PSA are associated with both localized and metastatic prostate cancer (CaP). However, PSA levels can change for many reasons other than cancer. Two common causes of high PSA levels in the absence of cancer are enlargement of the prostate (benign prostatic hypertrophy (BPH)) and infection in the prostate (prostatitis).

[0038] Thus, PSA is not a perfect test. Some men with prostate cancer do not have an elevated PSA, and most men with an elevated PSA do not have prostate cancer. Short of biopsy, no non-invasive tests provide a clear diagnosis of prostate cancer.

#### **Botanical compositions for prostate cancer therapy**

[0039] A botanical formulation presents an optimal first line therapy when high PSA levels are detected. botanical compositions, such as those disclosed herein, have very low toxicity and yet are effective against prostate cancer.

[0040] The botanical composition was designed following demonstration of a number of desirable functions among the ingredients and in the assembled composition. A number of therapeutically active chemical entities are present in *Ganoderma lucidum* (#9), *Scutellaria barbata* (#15), and *Salvia miltiorrhiza* (#14): ganoderic acid H, crytotanshinone, tanshinone IIA, scutellarin tetramethyl ether, scutellarin, apigenin and wogonin.

[0041] A number of chemical entities are present in the botanical composition: adenosine, ganoderic acid A, oleic acid, tanshinone IIA, scutellarin, apigenin, luteolin, and wogonin. Each of these chemical entities is known to demonstrate one or more of anti-viral, anti-inflammatory, immune modulatory, anti-angiogenic and anti-cancer/metastatic functions.

[0042] An exemplary combination of *Ganoderma lucidum* (#9), *Scutellaria barbata* (#15), and *Salvia miltiorrhiza* (#14) was designated OMN54, based on the synergism displayed by certain combinations of extracts of the three botanicals when each botanical is between 1% w/w and 90% w/w of the combined composition. Extracts of the botanicals were preferably made in

organic medium, such as alcohol, and non-alcoholic media including ester, lipid and the like. In a preferred embodiment the extracts were made in ethyl acetate medium.

**[0043]** Significant synergism was expressed by botanical compositions comprised of the following three organic extracts at the specified amounts (w/w):

**[0044]** (i) *Ganoderma lucidum* at 33-50% w/w. More specifically the *Ganoderma lucidum* extract is selected from 33%, 35%, 40%, 42%, 44%, 45%, 46%, 46.5%, 47%, 47.5%, 48%, 48.5%, 49%, 49.5% and 50%.

**[0045]** (ii) *Scutellaria barbata* at 33-50% w/w. More specifically the *Scutellaria barbata* extract is selected from 33%, 35%, 40%, 42%, 44%, 45%, 46%, 46.5%, 47%, 47.5%, 48%, 48.5%, 49%, 49.5% and 50%.

**[0046]** (iii) *Salvia miltiorrhiza* at 1-10% w/w. More specifically the *Salvia miltiorrhiza* extract is selected from 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5% and 10%.

**[0047]** In vitro studies have indicated that OMN54 did not modulate pro-inflammatory proteins in unstimulated PBMC (suggesting safety for long-term use). In the presence of inflammatory stimulus (PHA mitogen), OMN54 suppressed inflammatory signaling (significant anti-inflammatory activity). The effect of OMN54 in stimulated and unstimulated cells was observed for a number of proteins comprising cytokines, chemokines and growth factors.

**[0048]** The human prostate cancer cell line, LNCaP, is androgen sensitive and PSA positive. OMN54 was tested for activity on LNCaP cell line, on the expression of specific genes associated with prostate cancer. High levels of Prostate Specific Antigen (PSA) are associated with prostate cancer. The significant suppression of the PSA transcript by OMN54 is considered beneficial in the treatment of prostate cancer.

**[0049]** OMN54 displays profound antiproliferative effect on prostate cancer cells, inducing the apoptosis of both androgen receptor (AR)-positive (LNCaP) and AR-negative (DU-145) prostate cancer cell lines. OMN54 also displays NF-kappa B inhibition. The anti-inflammatory and anti-proliferative functions displayed by the botanical composition also are effective against BPH. Thus, following detection of high PSA levels and prior to invasive tests for diagnosis of cancer, a low toxicity botanical formulation which is effective against both BPH and neoplastic states like prostate cancer and PIN, provides a promising regimen for first intervention.

**[0050]** Other botanicals effective against prostate cancer include capsaicin, found in red peppers, which has a profound anti-proliferative effect on human prostate cancer cells in culture

and in mouse xenografts. (Mori A et al. Cancer Res. 2006 Mar 15;66(6):3222-9). Capsaicin down-regulates PSA expression by direct inhibition of PSA transcription mediated by inhibition of NF-kappa B activation by preventing its nuclear migration. (*Id.*)

[0051] A 2006 study of green tea derivatives demonstrated prostate cancer prevention in patients at high risk for the disease. (Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A (2006). "Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study". Cancer Res 66 (2): 1234–40). A phytochemical called di-indolyl-methane found in cruciferous vegetables is suspected of having anti-androgenic and immune modulating properties.

[0052] Botanicals are a valuable resource for the discovery and development of novel, naturally derived agents to treat human disease. Botanical extracts usually comprise multiple molecules and possess multiple functions useful in the treatment and prevention of disease. Botanical extracts also can function to maintain normal tissue homeostasis by affecting multiple biological pathways such as the inflammatory pathway, the immune response pathway and the oxidative stress response pathway. As a result, botanical extracts can alleviate the harmful side effects of many therapeutic agents used to treat multiple disease targets.

[0053] Botanicals have been demonstrated to be a successful source of anticancer compositions. Examples include *Gynostemma pentaphyllum* extract, *Camellia sinensis* (green tea) and *Crataegus pinnatifida* (hawthorn berries) and a method of making the same are the subject of U.S. Pat. Nos. 5,910,308 and 6,168,795. Some drugs, derived from plants that are currently used in cancer therapy were designed to perturb microtubule shortening (depolymerization) or lengthening (polymerization), such as paclitaxel, docetaxel, etoposide, vincristine, vinblastine, and vinorelbine (Compton, D. A., et al., (1999) Science 286:913-914). They share a common mechanism of action of binding to tubulin, the molecule of which microtubules are composed. (Compton, D. A., et al., (1999) Science 286:913-914). At least six plant-derived anticancer agents have received FDA approval (e.g., taxol, vinblastine, vincristine, topotecan, etoposide, teniposide). Other agents are being evaluated in clinical trials (e.g., camptothecin, 9AC, and irinotecan). Botanical extracts for the treatment cancer are described in U. S. Pat. Application Publications 20050214394 A1, 20050208070 A1 and 20050196409 A1.

[0054] The present invention provides novel compositions comprising botanical extracts to treat human diseases that are associated with multiple biological pathways in their pathologies. The compositions of the invention are comprised of two or more botanical extracts which work synergistically to modulate multiple biological pathways including but not limited to

inflammatory responses, immune responses, oxidative responses, viral and microbial infections, and cell proliferative responses.

**[0055]** (i) *Ganoderma lucidum* (Reishi): *Ganoderma lucidum* was praised for its effect of increasing memory and preventing forgetfulness in old age reported in Shen Nong Ben Cao Jing vol. 1 as early as 456-536 AD. Research on mice using orally or topically administered *Ganoderma lucidum* suggests that *Ganoderma lucidum* has anti-inflammatory activity. (Stavinoha, W., et al., (1995). Study of the anti-inflammatory efficacy of *Ganoderma lucidum*. In B.-K. Kim, & Y.S. Kim (Eds.), Recent Advances in *Ganoderma lucidum* research (pp. 3-7). Seoul Korea: The Pharmaceutical Society of Korea).

**[0056]** Applications of *Ganoderma* for (1) chemoprophylaxis of cancer in individuals at high risk for developing cancer (2) adjuvant use in the prevention of metastasis or recurrence of cancer (3) palliation of cancer related cachexia and pain and (4) adjunctive use with concurrent chemotherapy to reduce side-effects, maintain leukocyte counts and allow a more optimal dosing of chemo or radio therapeutics has been suggested (Chang, R. (1994) Effective Dose of *Ganoderma* in Humans; Proceedings of Contributed Symposium 59A, B 5th International Mycological Congress, Vancouver: pp. 117-121). Since studies of human dosage were traditional and empirical, a proper dose range of *Ganoderma* for therapy was calculated using this data and pharmacokinetic principals. The calculations suggested that a (1) *Ganoderma* dried fruit body dose of 0.5 to 1 g per day for health maintenance (2) 2 to 5 g per day if there is chronic fatigue, stress, auto immune, or other chronic health problems (3) 5 to 10 g per day for serious illness. (Chang, R. (1993) Limitations and Potential applications of *Ganoderma* and related fungal polyglycans in clinical ontology; First International Conference on Mushroom Biology and Mushroom products: 96).

**[0057]** While *Ganoderma lucidum* is preferred, one skilled in the art would recognize that other species of *Ganoderma* may also be used in the present invention. For example, *G. tsugae* has been shown to modulate Th1/Th2 and macrophage responses in allergic murine model, and recombinantly expressed fungal immunomodulatory protein, FIP-gts, from *G. tsugae* inhibited telomerase activity in A549 human lung adenocarcinoma cell line (Lin, J.Y. et al., (2006) Food Chem. Toxicol.; Liao, C.H. et al., (2006) Mo. Carcinog. 45(4):220-9). Examples of other species of *Ganoderma* include, but are not limited to, *G. applanatum*, *G. mongolicum*, *G. microsporum*, *G. subamboinense*, *G. pfeifferi*, *G. meredithae*, *G. oregonense* (*G. oregonse*), *G. resinaceum*, *G. oerstedii*, *G. unguatum*, *G. mirabile*, *G. tsugae*, *G. sessile*, *G. valesiacum*, *G. fornicatum*, *G. carnosum*, *G. australe*, and *G. boninense*.

[0058] (ii) *Scutellaria barbata* (Skullcap): *Scutellaria barbata*, a traditional Chinese medicine for liver, lung and rectal tumors, has been shown to inhibit mutagenesis, DNA binding and metabolism of aflatoxin B1 (AFB1) and cytochrome P450-linked aminopyrine N-demethylase (Wong B.Y. et al., (1993) Eur. J. Cancer Prev. 2(4):351-6; Wong B.Y. et al., (1992) Mutat. Res. 279(3):209-16). *Scutellaria barbata* is also capable of enhancing macrophage function in vitro and inhibiting tumor growth in vivo (Wong B.Y. et al., (1996) Cancer Biother. Radiopharm. 11(1):51-6).

[0059] This herb contains vitamins C and E as well as calcium, potassium, magnesium, iron, zinc scutellarin, volatile oil, tannin and bitter principles. The scutellarin acts on the central nervous system. Scutellarin, an active ingredient from *Scutellaria barbata* has been purified by liquid chromatography (Wenzhu Zhang et al., (2003) J. of Liquid Chromatography & Related Technologies 26 (13):2133-40).

[0060] (iii) *Scutellaria baicalensis*: *Scutellaria baicalensis* has been shown to have anti-proliferative and apoptotic activities against lymphocytic leukemia, lymphoma, and myeloma cell lines and possess anti-cancer activity on human malignant brain tumor cells (Kumagai, T. et al. (2006) Leuk. Res.; Scheck, A.C. et al., (2006) BMC Complement Altern. Med. 6:27).

[0061] *Scutellaria barbata* should not be confused with *Scutellaria baicalensis*. Banzhilian, the whole plant of *Scutellaria barbata*, should not be confused with "scute," the common name referring to huangqin, the root of *Scutellaria baicalensis*. "Although both are of the same genus, *Scutellaria barbata*, for which the tops are used, has essential oils among the active components, while *Scutellaria baicalensis* relies primarily on flavonoids, particularly baicalin and baicalein. *Scutellaria radix* (root of *Scutellaria baicalensis*) and *Scutellaria barbata* comprise different sets of flavonoids and show different effects on proliferation of human leukemia cell line HL-60. Sonoda et al., *J. Ethnopharm* 91:65-68 (2004)

[0062] While *Scutellaria barbata* and *Scutellaria baicalensis* are preferred, one skilled in the art would recognize that other species of *Scutellaria* may also be used in the present invention. For example, *Scutellaria radix* has been shown to suppress ethanol-induced caspase-11 expression and cell death in N(2)a cells, and Baicalein, a component of *Scutellaria radix*, leads to suppression of proliferation and induction of apoptosis in human myeloma cells (Kang, K. et al., (2005) Brain Res. Mol. Brain Res. 142(2):139-45; Ma, Z. et al. (2005) Blood 105(8):3312-8). Examples of other species of *Scutellaria* include, but are not limited to, *Scutellaria amabilis*, *Scutellaria radix*, *Scutellaria rehderiana*, and *Scutellaria lateriflora*. Preferred combinations are those where the extract from a particular species acts in synergy with extracts from other botanicals in the formulation or with other therapeutic agents in the composition.

[0063] (iv) *Salvia miltiorrhiza* (Dan Shen): There are over 900 species of salvia and many of them have histories of medicinal uses. Dan shen is used in traditional Chinese medicine to promote blood circulation and to remove blood stasis (Bensky D., Gamble *A Chinese herbal Medicine Materia Medica* 1987 Eastland Press: Seattle. 384). It increases the activity of SOD in platelets, thus providing protection against pulmonary embolism and inhibition of platelet aggregation. (Wang, X. et al., (1996) *Zhongguo Zhong Yao Za Zhi* 21:558-60). *Salvia miltiorrhiza* has been shown to lower cholesterol, reduce endothelial damage and to inhibit lipid peroxidation in hypercholesterolemic animals. This inhibition of oxidation of LDL may reduce atherosclerosis (Wu Y.J. et al., (1998) *Arteriosclerosis Thromb Vasc Biol* 18:481-6). A *Salvia miltiorrhiza* constituent has been found to inhibit noradrenalin-induced contraction of the aortic strips through reduction in  $Ca^{2+}$  mobilization. This vasodilatory activity may explain the traditional use of *Salvia miltiorrhiza* in hypertension (Nagai M. et al., *Biol Pharm Bull* (1996) 19:228-32). *Salvia miltiorrhiza* has been shown to have a markedly superior effect to nitroglycerin, with a more persistent action and better improvement of cardiac function (Bai, Y.R. and Wang, S.Z., (1994) *Zhongguo Zhong Xi Yi Jie He Za Zhi* 14:24-5, 4).

[0064] *Salvia miltiorrhiza* is also the top ingredient in Dan Shen Compound. Dan Shen Compound comprises four important herbs for the improvement of peripheral circulation and general wellbeing. The actions of *Crataegus levigata* are enhanced by the Chinese herb *Salvia miltiorrhiza* (Dan Shen), the Indian herb *Coleus forskohlii* and *Valeriana officinalis*. Chinese herbal medicine utilizes *Salvia miltiorrhiza* for women's irregularities, abdominal pain, insomnia, hives, hepatitis and mastitis.

[0065] (v) *Hippophae rhamnoides* (sea buckthorn): Sea buckthorn seed oil contains a high content of the two essential fatty acids, linoleic acid and  $\alpha$ -linolenic acid, which are precursors of other polyunsaturated fatty acids such as arachidonic and eicosapentaenoic acids. The oil from the pulp/peel of seabuckthorn berries is rich in palmitoleic acid and oleic acid (Chen et al., "Chemical composition and characteristics of seabuckthorn fruit and its oil." *Chem. Ind. Forest Prod. (Chinese)* 10 (3), 163-175). The increase in the level of  $\alpha$ -linolenic acid in plasma lipids showed a clear improving effect on AD symptoms (Yang et al., (2000) *J. Nutr Biochem.* 11(6):338-340). These effects of  $\alpha$ -linolenic acid may have been due to both changes in the eicosanoid composition and other mechanisms independent of eicosanoid synthesis (Kelley (1992) *Nutrition*, 8 (3), 215-2).

[0066] Antioxidant and immunomodulatory properties of sea buckthorn (*Hippophae rhamnoides*) have been demonstrated using lymphocytes as a model system. (Geetha et al. *J Ethnopharmacol* 2002 Mar; 79(3):373-8). The antiulcerogenic effect of a hexane extract from



*Hippophae rhamnoides* has also been demonstrated. (Suleyman H. et al., (2001) *Phytother Res* 15(7):625-7). Radioprotection by an herbal preparation of *Hippophae rhamnoides* against whole body lethal irradiation in mice suggests free radical scavenging, acceleration of stem cell proliferation and immunostimulation properties. (Goel H.C. et al., (2002) *Phytomedicine* 9(1):15-25)

[0067] (vi) *Camellia sinensis* (Green tea): Dried leaves from the *Camellia sinensis* plant is processed into three types of tea: oolong tea, black tea, and green tea. Green tea extract is a bioflavonoid-rich, potent extract which is used primarily for fighting free radicals. It has a high content of polyphenols, which are a type of bioflavonoids. In making green tea, the tea leaves are stabilized by moist or dry heat which destroys the enzyme polyphenoloxidase and thus, prevents oxidation of polyphenols. These polyphenols are the main biologically active ingredients in green tea. In preferred embodiments, the green tea is Dragon Well tea or Lung Ching tea.

[0068] The polyphenols in green tea are catechins, with multiple linked ring-like structures. Polyphenols are a form of bioflavonoids with several phenol groups. They control both taste and biological action. Catechins, a chemical group of polyphenols possessing antioxidant properties (protecting cells from free radical-mediated damage), include epigallocatechin-3 gallate (EGCG), epigallocatechin, and epicatechin-3-gallate. Recently, EGCG has been shown to be an inhibitor of urokinase (Jankun et al., (1997) *Nature* 387:561), and quinol-oxidase; enzymes that may be crucial for growth of tumor cells. Epigallocatechin-3 gallate (EGCG) also protects against digestive and respiratory infections.

[0069] Novel tumor inhibiting, immune boosting, inflammation reducing and anti-oxidative properties observed for compositions comprising a combination of two or more extracts of *Ganoderma lucidum*, *Scutellaria barbata*, *Scutellaria baicalensis*, and *Salvia miltiorrhiza* and, optionally, *Hippophae rhamnoides* (seabuckthorn) and *Camellia sinensis* (green tea) and the synergistic effects demonstrated by novel combinations of two or more of these extracts used in the method according to the present invention are a likely result of combinations of one or more of saponins, flavonoids, and polyphenols present in the extracts.

#### **Formulations of Botanical Compositions**

[0070] The compositions of the present invention can be in any form which is effective, including, but not limited to dry powders, grounds, emulsions, extracts, and other conventional compositions. To extract or concentrate the effective ingredients of the compositions, typically the botanical part is contacted with a suitable solvent, such as water, alcohol, methanol, mixed solvents, or any other solvents. The choice of the solvent can be made routinely, e.g., based on

the properties of the active ingredient that is to be extracted or concentrated by the solvent. Preferred active ingredients of the compositions *crenulata* include, but are not limited to, salidroside, tyrosol,  $\beta$ -sitosterol, gallic acid, pyrogallol, crenulatin, rhodionin, and/or rhodiosin. These ingredients can be extracted in the same step, e.g., using an alcoholic solvent, or they may be extracted individually, each time using a solvent which is especially effective for extracting the particular target ingredient from the plant. In certain embodiments, extraction can be performed by the following process: Milling the selected part, preferably root, to powder. The powder can be soaked in a desired solvent for an amount of time effective to extract the active agents from the compositions. The solution can be filtered and concentrated to produce a paste that contains a high concentration of the constituents extracted by the solvent. In some cases, the paste can be dried to produce a powder extract of the compositions *crenulata*. The content of active ingredient in the extract can be measured using HPLC, UV and other spectrometry methods.

**[0071]** The compositions of the present invention can be administered in any form by any effective route, including, e.g., oral, parenteral, enteral, intraperitoneal, topical, transdermal (e.g., using any standard patch), ophthalmic, nasally, local, non-oral, such as aerosol, inhalation, subcutaneous, intramuscular, buccal, sublingual, rectal, vaginal, intra-arterial, and intrathecal, etc. It can be administered alone, or in combination with any ingredient(s), active or inactive, including in a medicinal form, or as a food or beverage additive.

**[0072]** In preferred embodiments of the invention, the compositions are administered orally in any suitable form, including, e.g., whole plant, powdered or pulverized plant material, extract, pill, capsule, granule, tablet or a suspension.

**[0073]** The compositions can be combined with any pharmaceutically acceptable carrier. By the phrase, "pharmaceutically acceptable carriers," it is meant any pharmaceutical carrier, such as the standard carriers described, e.g., Remington's *Pharmaceutical Science*, 18th Edition, Mack Publishing company, 1990. Examples of suitable carriers are well known in the art and can include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solutions, phosphate buffered saline containing Polysorb 80, water, emulsions such as oil/water emulsion and various types of wetting agents. Other carriers may also include sterile solutions, tablets, coated tablets pharmaceutical and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols. Such carriers can also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods. Generally

excipients formulated with the compositions are suitable for oral administration and do not deleteriously react with it, or other active components.

**[0074]** Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose and the like. Other additives include, e.g., antioxidants and preservatives, coloring, flavoring and diluting agents, emulsifying and suspending agents, such as acacia, agar, alginic acid, sodium alginate, bentonite, carbomer, carrageenan, carboxymethylcellulose, cellulose, cholesterol, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, octoxynol 9, oleyl alcohol, povidone, propylene glycol monostearate, sodium lauryl sulfate, sorbitan esters, stearyl alcohol, tragacanth, xanthan gum, and derivatives thereof, solvents, and miscellaneous ingredients such as microcrystalline cellulose, citric acid, dextrin, dextrose, liquid glucose, lactic acid, lactose, magnesium chloride, potassium metaphosphate, starch, and the like.

**[0075]** The botanical compositions can also be formulated with other active ingredients, such as anti-oxidants, vitamins (A, C, ascorbic acid, B's, such as B1, thiamine, B6, pyridoxine, B complex, biotin, choline, nicotinic acid, pantothenic acid, B12, cyanocobalamin, and/or B2, D, D2, D3, calciferol, E, such as tocopherol, riboflavin, K, K1, K2). Preferred compounds, include, e.g. creatine monohydrate, pyruvate, L-Carnitine,  $\alpha$ -lipoic acid, Phytin or Phytic acid, Co Enzyme Q10, NADH, NAD, D-ribose, amino acids such as L-glutamine, Lysine, chrysin; pre-hormones such as 4-androstenedione, 5-androstenedione, 4(or 5-)androstenediol, 19-nor-4 (or 5-)androstenedione, 19-nor-4 (or 5-)androstenediol, Beta-ecdysterone, and 5-Methyl-7-Methoxy Isoflavone. Preferred active ingredients include, e.g., pine pollen, fructus lycii, *Hippophae rhamnoides*, Ligusticum, Acanthopanax, Astragalus, Ephedra, codonopsis, polygola tenuifolia Willd, Lilium, Sparganium, ginseng, panax notogiseng, Garcinia, Guggule, Grape Seed Extract or powder, and/or Ginkgo Biloba.

**[0076]** Other plants and herbs which can be formulated with the compositions of the present invention includes those mentioned in various text and publications, e.g., E.S. Ayensu, Medicinal Plants of West Africa, Reference Publications, Algonac, Mich. (1978); L. Boulos, Medicinal Plants of North Africa, Reference Publications Inc., Algonac, Mich. (1983); and N. C. Shah, (1982) J. Ethnopharm, 6:294-5.

**[0077]** A botanical formulation may comprise biologics and chemical entities, in addition to or in the place of, botanical extracts. Examples of biologics that may comprise a botanical

composition include but are not limited to blood and blood products, cells, tissues and organs, gene therapy vectors, viral and bacterial vaccines, therapeutic products produced through biotechnology such as antibodies, monoclonal antibodies, and the like.

**[0078]** Pharmaceutically active agents that can comprise a botanical composition include, but are not limited to antioxidants, anticarcinogens, anti-inflammatory agents, hormones and hormone antagonists, anti-hypertensive agents, anti-inflammatory agents, tranquilizers, cardiogenic agents, antidepressants, corticosteroids, anti-ulcer agents, anti-allergy agents and anti-obesity agents, antibiotics, antibacterial agents, bacterial agents, and other medically useful drugs such as those identified in, e.g., Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Company, 1990. A preferred composition of the present invention comprises, about 1%-100%, preferably about 20-70% of the botanical extract and, optionally, a pharmaceutically-acceptable excipient. Another preferred composition of the present invention comprises, about 1%-99%, preferably about 20-70% of botanical extracts, 0.1-99%, preferably 1-10% of one or more pharmaceutically active agents and, optionally, a pharmaceutically-acceptable excipient.

**[0079]** In some embodiments, the botanical composition comprises a chemotherapeutic agent either in a single formulation or separately administered as part of a therapeutic regimen.

**[0080]** According to the instant invention, it has been surprisingly observed that concurrent treatment with Bicalutamide and OMN54 results in more effective remediation of prostate cancer symptoms with significantly reduced side effects and synergistic anticancer activity.

**[0081]** Bicalutamide is used in monotherapy or with another medication (luteinizing hormone-releasing hormone [LHRH]; such as leuprolide or goserelin) to treat metastatic prostate cancer (cancer that started in the prostate and has spread to other parts of the body).

Bicalutamide is a nonsteroidal antiandrogen, which works by blocking the effect of androgen (a male hormone), to stop the growth and spread of cancer cells. Bicalutamide comes as a tablet to take by mouth. It is usually taken with or without food once a day, either in the morning or evening. Standard dosages of bicalutamide are dosages of 25, 50, and up to 150 mg single dose per day. For combination therapy with LHRH, bicalutamide therapy is started on the same day you begin injecting the luteinizing hormone-releasing hormone.

**[0082]** The instant invention relates to the administration of bicalutamide and the botanical compositions (such as OMN54) concurrently. By concurrently, it is understood that the botanical composition is administered simultaneously, on the same day, or within 24 hours of bicalutamide.

**[0083]** In some embodiments, the botanical composition and bicalutamide is formulated as a tablet dosage form comprising: a) a first layer comprising a tablet of bicalutamide, wherein the tablet is inlaid in the first layer with one or more other pharmaceutically acceptable excipients; and b) a second layer comprising a composition comprising two or more extracts of *Ganoderma lucidum*, *Scutellaria barbata*, *Scutellaria baicalensis*, *Salvia miltiorrhiza*, and optionally other pharmaceutically acceptable excipients. In some embodiments, the dosage form comprises a bilayered dosage form. In some aspects the tablet is coated with one or more enteric polymers, pharmaceutically acceptable seal coat polymers or rate controlling polymers. In some aspects, the two active ingredients are provided as a dispersion provided in a capsule, granule, mini-tablet or tablet form.

**[0084]** In some aspects, bicalutamide and/or the botanical extracts composition are present in an immediate release, delayed release, sustained release, extended release, controlled release or modified release form.

**[0085]** In other embodiments, the invention relates to a kit containing separate dosage forms for each active ingredient, for example, comprising bicalutamide provided in a tablet form and the botanical extracts provided in a capsule form. In some embodiments, the capsule and the tablet are provided in a single package (such as a blister pack).

**[0086]** The present invention relates to methods of administering the compositions, e.g., to provide anti-inflammatory effects, to reduce inflammation, to provide antioxidant effects, to protect against oxidation, to provide antiproliferative effects, to provide anti-cancer effects, to promote DNA repair, to provide anti-radiation effects, to protect against radiation, and other conditions and diseases as mentioned herein.

**[0087]** An effective amount of the compositions are administered to such a host. Effective amounts are such amounts which are useful to achieve the desired effect, preferably a beneficial or therapeutic effect as described above. Such amount can be determined routinely, e.g., by performing a dose-response experiment in which varying doses are administered to cells, tissues, animal models (such as rats or mice in maze-testing, swimming tests, toxicity tests, memory tests as performed by standard psychological testing, etc.) to determine an effective amount in achieving an effect. Amounts are selected based on various factors, including the milieu to which the virus is administered (e.g., a patient with cancer, animal model, tissue culture cells, etc.), the site of the cells to be treated, the age, health, gender, and weight of a patient or animal to be treated, etc. Useful amounts include, 10 milligrams-100 grams, preferably, e.g., 100 milligrams-10 grams, 250 milligrams-2.5 grams, 1 gm, 2 gm, 3 gm, 500 milligrams-1.25 grams. etc., per dosage of different forms of the compositions such as the botanical powder, botanical

extract paste or powder, tea and beverages prepared to contain the effective ingredients of the compositions, and injections, depending upon the need of the recipients and the method of preparation.

**[0088]** The liquid, pharmaceutically active formulation comprises a pharmaceutically active botanical composition in a liquid diluent or carrier. The active ingredient may be dissolved or dispersed in the liquid diluent or carrier, which may be a water miscible or water immiscible medium. Examples of liquid diluents or carriers include the following three classes: (a) Water miscible carriers: Propylene Glycol, Polyethylene Glycol, Water, Solketal, Glycofurol, Dimethylisorbide, Nonionic surface active agents; (b) Oils and Organic carriers: Fractionated Coconut Oil, Sesame Oil, Soya Bean Oil, Vegetable Oil, Liquid Paraffin, Isopropylmyristate, Triacetin; and (c) Semi-solid carriers: High molecular weight polyethylene glycols, and White soft paraffin.

**[0089]** In some embodiments, one or more emulsifiers or surfactants are included in the formulation. Suitable emulsifiers which can be used include one or more of fatty acids such as oleic acid, polyoxyethylene glycerol esters of fatty acids, such as Tagats; polooxylated castor oil, ethylene glycol esters, such as glycol stearate and distearate; propylene glycol esters, such as propylene glycol myristate; glyceryl esters of fatty acids, such as glyceryl stearates and monostearates; sorbitan esters, such as spans and tweens; polyglyceryl esters, such as polyglyceryl 4-oleate; fatty alcohol ethoxylates, such as Brij type emulsifiers; ethoxylated propoxylated block copolymers, such as poloxamers; polyethylene glycol esters of fatty acids, such as Labrafils, Labrafacs, and Labrasols; cremophores; glycerol monocaprylate/caprate, such as Campmul CM 10; Gelucire, Capryol, Captex, Acconon, transcitol, triacetin, and the like. In some embodiments, antioxidants and/or diluents are used in the formulation.

**[0090]** Compositions of the present invention comprise effective amounts of a combination of two or more extracts of *Ganoderma lucidum*, *Scutellaria barbata*, *Scutellaria baicalensis*, *Salvia miltiorrhiza*, and optionally, *Hippophae rhamnoides* (sea buckthorn) that exhibit synergy.

**[0091]** In one aspect of the invention, the botanical composition comprises effective amounts of extracts of *Ganoderma lucidum*, *Scutellaria barbata*, and *Salvia miltiorrhiza*. The dosage of the composition can be readily determined by one of skill in the art based on the effective concentrations of compositions shown to display the various properties described herein.

**[0092]** Compositions comprising different ratios of the individual extracts can similarly be determined. For example, a composition may exhibit anti-inflammatory effects at one concentration or ratios of combinations of extracts and varying degrees of cytotoxic effects at

other concentrations or ratios of combinations of extracts. Any ratio of extracts of two or more of *Ganoderma lucidum*, *Scutellaria barbata*, *Scutellaria baicalensis*, and *Salvia miltiorrhiza* can be used in the compositions of the invention. It is preferred that each extract is present in the composition in equal amounts or at about 1% to about 90% of the total composition. In some embodiments of the invention, a particular extract comprises at least 1%, 1.5%, 2%, 5%, 10%, 15%, 25%, 33%, 40%, 45%, 47.5%, 48.5%, 49.5%, 50%, 60%, 66%, 75%, 90% or 98% by weight of the composition. In one embodiment the OMN54 comprises about 1-3% *Salvia miltiorrhiza*, and approximately equal amounts (45-50%) of *Scutellaria barbata* and *Ganoderma lucidum*.

**[0093]** In a further embodiment, the compositions of the present invention comprise botanical compounds that are useful in compositions to be administered in conjunction with therapeutic agents for the treatment of disease. These compositions exhibit synergistic action with the therapeutic agent based on their anti-inflammatory, antioxidant, immune modulating, antiviral, antibacterial, antiproliferative activity or any combination of activities thereof.

**[0094]** The compositions demonstrate antioxidant activity which prevents damage to chromosomes/genes, reduces effect of mutagens, alleviates side-effects of chemotherapeutic agents, alleviates side-effects of hormone therapeutic agents, and enhances cell repair mechanisms.

**[0095]** The compositions further demonstrate immune system boosting activity which facilitates elimination of (i) damaged cells or (ii) cells with damaged genes. Further, the compositions provide general benefits of improving immune condition (passive immunotherapy).

**[0096]** The botanical sources of the extracts are botanicals that are essentially nontoxic with a long history of usage of the individual compounds/extracts. Anti-mutagenic properties as evidenced by Ames test results (together with increased sensitivity by synergism) reduce levels of chemotherapeutic agents necessary for treatment resulting in reduced toxicity for patients.

**[0097]** The botanical compositions demonstrate the ability to enhanced cell cycling which could make the botanical composition of the invention a powerful adjuvant to chemotherapy (e.g., with bicalutamide), hormonal therapy, or radiation therapy by increasing effectiveness and reducing necessary dosages of chemotherapeutic agents and hormone therapeutic agents.

**[0098]** Quality control. IC<sub>50</sub> based compositions can be standardized based on specific activities of defined properties.

[0099] The compositions are also suited for convenient (oral) drug delivery. Compositions are extracts made with hot water, alcoholic solvents (ethanol) and non-alcoholic organic solvents (ester, lipid, ethyl acetate, etc.).

[0100] Overall the botanical compositions show mostly cytostatic effect with very weak cytotoxic effects in the compositions of the invention. Histopathology of cells treated with the compositions of the invention indicates minimal retention of dead cancer cells which enhance recovery following cancer therapy.

### EXAMPLES

[0101] Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are illustrative only, and not limiting of the remainder of the disclosure in any way whatsoever.

[0102] The following combinations of extracts were used throughout the examples: *Ganoderma lucidum*, *Scutellaria barbata*, optionally *Scutellaria baicalensis* and *Salvia miltiorrhiza* extracts when combined in ratios that exhibit synergism are referred to as OMN54 in the specification.

[0103] In addition, the compositions of the invention may include, optionally, *Panax Quinquefolium* (Western ginseng), *Camellia sinensis* (green tea), and *Hippophae rhamnoides* (sea buckthorn).

[0104] One skilled in the art would appreciate that while the following examples are illustrative of the invention, any cell line may be used. For example, although not limiting, cells may be obtained from ATCC, Rockville, Md.

[0105] One skilled in the art would also appreciate that while the foregoing examples are illustrative of the invention, multiple prostate cancer in vivo models may be used. For example, CaP xenografts in mice may be utilized. Additionally, a Pten knockout mouse strain, in which heterozygous mice develop tumors of the uterus, prostate, thyroid, colon, and adrenal medulla, may be obtained from the Mouse Models of Human Cancers Consortium (Podsypanina K. et al., (1999) Proc. Nat'l Acad. Sci. USA 96: 1563-1568).

#### **Example 1: Methods for Preparation of Botanical Extracts**

[0106] The compositions of the present invention may be administered as dried herbs. Botanical preparations contain phytochemicals some of which are soluble in aqueous media while others are relatively more soluble in organic (alcohol, lipid) media. Different extraction methods were used and tested for the ability to extract effective ingredients from the herbs.



Extraction methods include: Aqueous (hot water) extraction; Organic (lipid fraction) extraction; non-alcoholic organic (ethyl acetate) extraction; and alcohol (ethanol) Extraction.

[0107] Products are prepared from herbs or herb blends by extraction with solvent (hot water, 80% ethanol, or ethyl acetate) under reflux for 30-60 minutes, separated by filtration to obtain a filtrate, and air dried for further analysis. The filtrates were combined, diluted or concentrated prior to determination of activities.

**Example 2: combination of OMN54 with Casodex<sup>®</sup> (bicalutamide) on Prostate Cancer Cells**

[0108] The combination of OMN54 with Casodex<sup>®</sup> (bicalutamide) achieved greater anti-tumor growth effect than either OMN54 or bicalutamide alone in C4-2 castration-resistant human prostate cancer cells *in vitro* (Figure 1). Treatment with either bicalutamide (10  $\mu$ M) or OMN54 (10  $\mu$ g/ml) alone had minimum inhibitory effect on C4-2 cells, however, the combination of OMN54 (10  $\mu$ g/ml) with bicalutamide (10  $\mu$ M) significantly inhibited cell growth at 48 h (see Figure 1).

[0109] The effects of these two agents and their combination on Androgen Receptor (AR) expression. The expression of AR protein was reduced in the OMN54 treatment group, but not in the control group. The AR expression was further decreased in the combination of OMN54 and Casodex<sup>®</sup> (bicalutamide) group compared to either the OMN54 or bicalutamide group alone (see Figure 2).

**Example 3: Effect of OMN54 and bicalutamide treatment on AR expression**

[0110] Figure 2 shows the effect of OMN54 and bicalutamide treatment on AR expression in C4-2 cells by Western blot analysis.

[0111] Using a clonogenic assay, it was found that the combination of OMN54 with Casodex<sup>®</sup> (bicalutamide) achieved greater anti-tumor growth effect than either OMN54 or bicalutamide alone in C4-2 castration-resistant human prostate cancer cells *in vitro* (Figure 3). In this assay, treatment with bicalutamide (20  $\mu$ M) demonstrated no effect, while OMN54 (50  $\mu$ g/ml) demonstrated significant inhibitory effect on C4-2 cells, however, the combination of OMN54 (50  $\mu$ g/ml) with bicalutamide (20  $\mu$ M) inhibited cell growth even further at 24 h (see Figure 3 and Figure 4).

**Example 4: Effect of OMN54 and bicalutamide treatment on clonogenic ability**

[0112] Figure 3 shows the effect of combination of OMN54 and bicalutamide on clonogenic ability in C4-2 cells. C4-2 cells were treated with OMN54 and bicalutamide either alone or together for 24 hr. The cells were then trypsinized and plated at 800 cells per dish and grown

for 2 weeks. The cells were fixed and stained. These data suggest that combination of OMN54 and bicalutamide synergistically inhibits castration-resistant prostate tumor growth.

[0113] Figure 4 also shows measurement of the effect of combination of OMN54 and bicalutamide on clonogenic ability in C4-2 cells.

[0114] The effects of OMN54 with Casodex<sup>®</sup> (bicalutamide) on androgen-sensitive LNCaP prostate cancer cell growth *in vitro*. As with previous studies, the combination of OMN54 with bicalutamide demonstrated more effect than either of the agents alone (see Figure 4).

**Example 4: Effect of OMN54 and bicalutamide treatment on LNCaP cell growth**

[0115] Figure 5 shows the effect of combination of OMN54 and bicalutamide on LNCaP cell growth. LNCaP cells were treated with OMN54 and bicalutamide either alone or combination for 48 hr. These pictures show that combination of OMN54 and bicalutamide induces apoptosis, while OMN54 or bicalutamide alone mostly inhibits cell growth.

[0116] This experimental evidence suggests that further inhibition of Androgen Receptor (AR) signaling by combining OMN54 (which reduces AR protein expression) and anti-androgen bicalutamide (which blocks ligand binding to AR or reduces ligand levels) may achieve better anti-cancer efficacy than anti-androgen therapy alone for castration-resistant prostate cancer. Additionally, it is now demonstrated that the combination of these two agents appears to have greater therapeutic effect on LNCaP androgen-sensitive prostate cancer cells than either agent alone.

**Example 5: Establishment of a human prostate cancer tissue xenograft/mouse model**

[0117] A more predictive human tumor tissue xenograft model was used to assess efficacy of the botanical compositions. (Clin Cancer Research 2006: 12(13); 4043-4054) Human tumor grafts are better than cell line injections and human tumors grafted on kidney capsule have more immediate blood supply than subcutaneous grafts, thus more biological diversity is retained. The xenograft model more closely resembles clinical cancer than traditional *in vivo* models

[0118] Preclinical testing of prostate cancer therapeutics has been largely carried out using xenograft models in which human prostate cancer cell lines have been subcutaneously injected into immunodeficient mice. However, cancer cell xenografts may not accurately mimic the behavior of prostate tumors *in vivo*. In fact, cancer cell line xenograft models have a poor record of accurately predicting the clinical efficacy of anticancer agents. A novel xenograft model was established for a variety of pre-cancerous and cancerous human tissues, including prostate cancer tissue. Most importantly, the xenografts in the model retain the histological characteristics of the parental tissue. For selected types of cancer that the xenografts respond to

therapy in a manner similar to that observed in patients. For example, prostate cancer tissue grown in SCID mice showed a dramatic response to androgen ablation therapy as regularly found in the clinic.

[0119] Xenografts of human prostate cancer cell line DU145 were grown in vivo. One of two tissue xenografts grew to the size of a walnut. Tumors are grafted to the renal site survive and retain their original histopathology and differentiation marker profile, even after serial passages. The prostate cancer tissue very rapidly grows in SCID mice with a doubling time about 5 days. Cytogenetic analyses show some abnormal chromosomes. Not only are there translocations, there are also deletions and duplication of chromosomal segments (Note: since each chromosome has its own display color, more than one color along the length of a chromosome indicates a translocation). The Spectral Karyotyping (SKY) analysis shows that the tissue of a DU145 cancer xenograft contained only a low number of karyotypic alterations, although the cancer is highly advanced.

**Example 6: Efficacy study on prostate cancer cell line (DU145)**

[0120] Tumor xenografts from a prostate cell line DU145 were cut into 2 mm<sup>3</sup> pieces and grafted into SCID/nod mice. Treatment was started at day 13 (mean volume = 15.6 mm<sup>3</sup>). The mice were divided into 3 equal groups for treatment with saline; *Ganoderma lucidum*, *Salvia miltiorrhiza*, and *Scutellaria barbata* at 3.3 IC<sub>50</sub>; and estramustine sodium phosphate (EMCYT<sup>®</sup>) and docetaxel (E+D). The combination of *Ganoderma lucidum*, *Salvia miltiorrhiza*, and *Scutellaria barbata* showed a significant inhibitory effect comparable to the E+D regimen. OMN54 significantly inhibited growth of DU145 (androgen-independent) human prostate cancer tumors in vivo. Importantly, this effect on tumor volume was comparable to that of standard chemotherapy which is associated with significant toxicities to patients.

**Example 7: In vivo anti-prostate tumor activity assay**

[0121] 6-week old male nude mice (BALB/c-nu/nu) are used for the experimental animal model. Animals are cut open from the abdomen and inoculated with the human prostate cancer cell lines-LNCaP cells (2x 10<sup>6</sup> cells/50μl/Hanks Buffered Saline Solution/mice) from back side of the prostate with a 30g needle. The cut abdomens are then sutured with 5-0 thread. After 2 weeks with regular feeding, blood is drawn from each animal for measuring the serum PSA value. The compositions of the invention are administered orally to the mice as follows; 3 groups of 6 mice received 43.65, 14.4, or 4.3 mg/animal/day for 21 days. Age-matched control mice are treated with saline for the same period.

[0122] Grafts are then harvested to determine the effect on tumor volume, histology, apoptosis index (Tunel assay) and proliferation index (proliferation marker Ki67 staining). The TUNEL

assay detects apoptosis-induced DNA fragmentation through a quantitative fluorescence assay. Terminal deoxynucleotidyl transferase (TdT) catalyzes the incorporation of bromo-deoxyuridine (BrdU) residues into the fragmenting nuclear DNA at the 3'-hydroxyl ends by nicked end labeling. A TRITC-conjugated anti-BrdU antibody can then label the 3'-hydroxyl ends for detection.

**[0123]** All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

**[0124]** Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

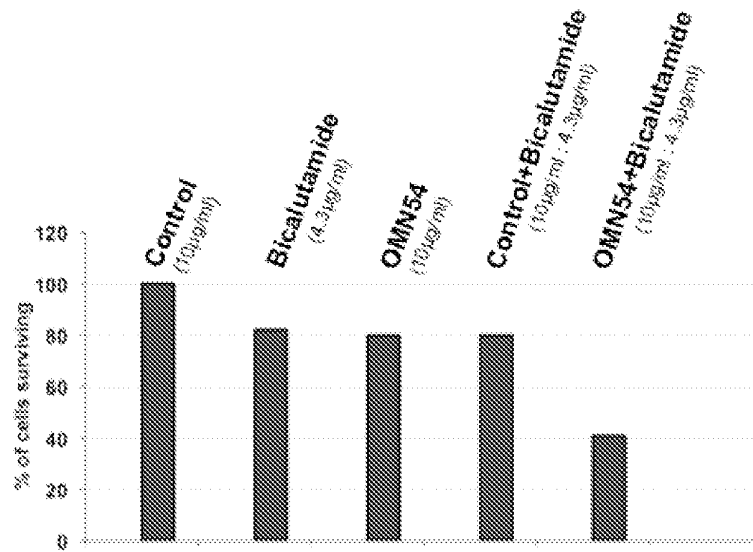
## THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for treatment or reducing the severity of prostate cancer in a subject, the method comprising:
  - administering an effective amount of a botanical composition comprising two or more extracts in organic medium of *Ganoderma lucidum*, *Salvia miltiorrhiza*, and *Scutellaria barbata*, wherein each extract comprises from about 1 to about 90 percent w/w and wherein the botanical composition is effective for reducing androgen receptor protein expression; and
  - co-administering an effective amount of bicalutamide,
  - wherein the co-administration of bicalutamide and the botanical composition achieves more effective therapy than administration of either agent alone.
2. The method of claim 1, wherein the bicalutamide is administered at 25, or 50 or 150 mg per day.
3. The method of claim 1 or claim 2, wherein the co-administration is administration of the botanical composition and the administration of bicalutamide is between 0 and 24 hours apart.
4. The method of any one of claims 1 to 3, wherein the extract is a non-alcoholic organic extract.
5. The method of claim 4, wherein the extract is made with ethyl acetate ester.
6. The method of any one of claims 1 to 5, wherein co-administration of bicalutamide and the botanical composition results in reduced toxicity and side-effects compared to administration of bicalutamide alone.
7. The method of any one of claims 1 to 6, further comprising administration of a luteinizing hormone releasing hormone (LHRH) to the subject.
8. The method of claim 1 or claim 2, wherein bicalutamide and the botanical composition are administered in a single dosage form.
9. The method of claim 1 or claim 2, wherein bicalutamide and the botanical composition are administered in a plurality of dosage forms.

10. The method of claim 1 or claim 2, wherein bicalutamide and the botanical composition are administered orally.
11. The method of any one of claims 1 to 10, wherein the prostate cancer is a castration-resistant prostate cancer.
12. The method of any one of claims 1 to 10, wherein the prostate cancer is an androgen-sensitive prostate cancer.
13. The method of claim 1 or claim 2, wherein bicalutamide is administered in a sub-therapeutic dose.
14. A tablet dosage form comprising:
  - a) a first layer comprising a tablet of bicalutamide, wherein the tablet is inlaid in the first layer with one or more other pharmaceutically acceptable excipients; and
  - b) a second layer comprising a composition comprising two or more organic extracts of *Ganoderma lucidum*, *Scutellaria barbata*, *Scutellaria baicalensis*, *Salvia miltiorrhiza*, and optionally other pharmaceutically acceptable excipients.
15. The dosage form of claim 14, comprising a bilayered dosage form.
16. The dosage form of claim 14, wherein the tablet is coated with one or more enteric polymers, pharmaceutically acceptable seal coat polymers or rate controlling polymers.
17. The dosage form of any one of claims 14 to 16 that comprises the botanical composition, comprising an effective amount of *Ganoderma lucidum* extract selected from 33%, 35%, 40%, 42%, 44%, 45%, 46%, 46.5%, 47%, 47.5%, 48%, 48.5%, 49%, 49.5% and 50%.
18. The dosage form of any one of claims 14 to 16 that comprises the botanical composition, comprising an effective amount of *Scutellaria barbata* extract selected from 33%, 35%, 40%, 42%, 44%, 45%, 46%, 46.5%, 47%, 47.5%, 48%, 48.5%, 49%, 49.5% and 50%.

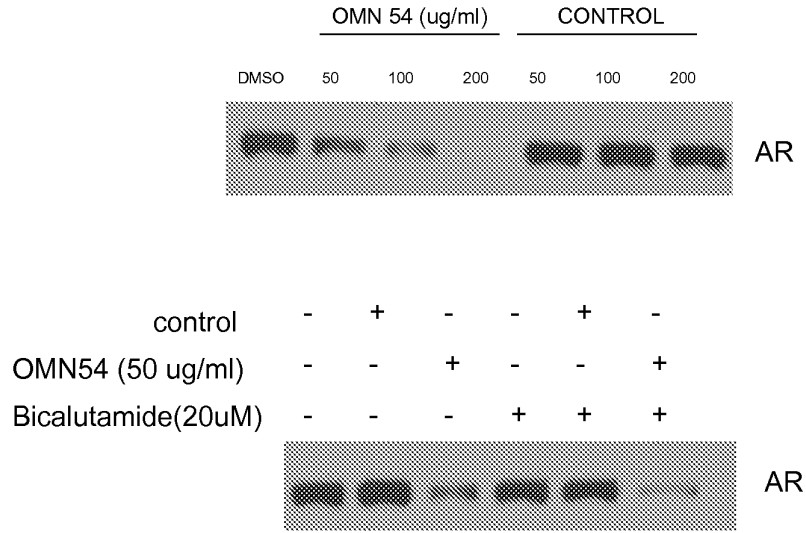
19. The dosage form of any one of claims 14 to 16 that comprises the botanical composition, comprising an effective amount of *Salvia miltiorrhiza* extract selected from 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5% and 10%.
20. Use of a botanical composition comprising two or more extracts in organic medium of *Ganoderma lucidum*, *Salvia miltiorrhiza*, and *Scutellaria barbata*, wherein each extract comprises from about 1 to about 90 percent w/w and wherein the botanical composition is effective for reducing androgen receptor protein expression and an effective amount of bicalutamide in the preparation of a medicament for treatment or reducing the severity of prostate cancer when the botanical composition and bicalutamide are co-administered such that they achieve more effective therapy than administration of either agent alone.

FIGURE 1





**FIGURE 2**



**FIGURE 3**

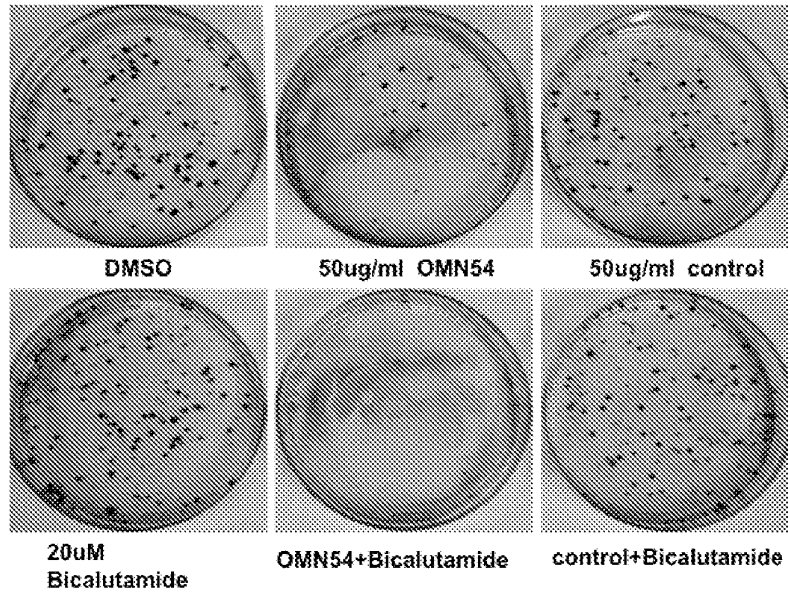


FIGURE 4

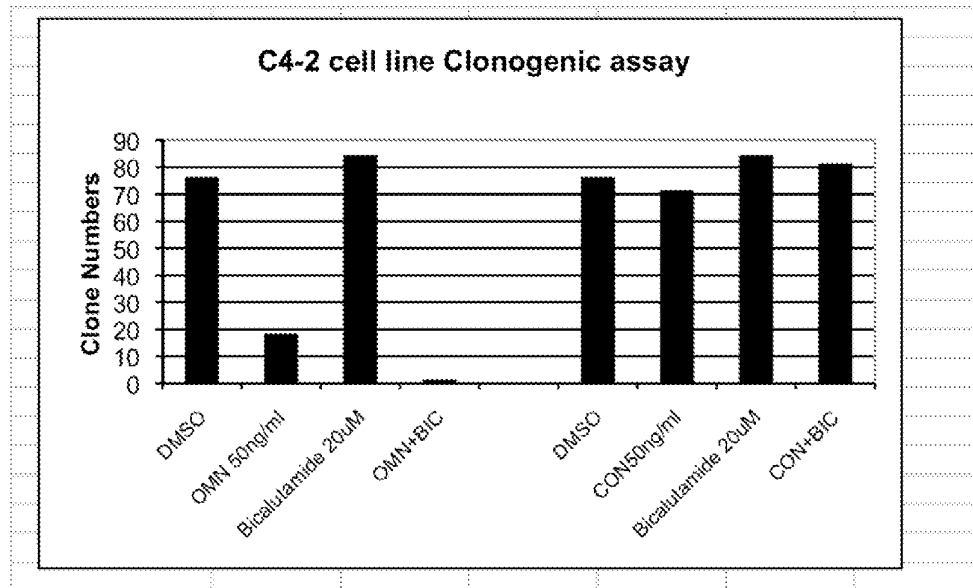


FIGURE 5

