



**COMPOSITION COMPRISING XANTHOCERAS SORBIFOLIA
EXTRACTS, COMPOUNDS ISOLATED FROM SAME, METHODS
FOR PREPARING SAME AND USES THEREOF**

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This application claims benefit of U.S. Serial Nos. 60/509,851, Filed October 9, 2003 and U.S. Serial No. 60/532,101, Filed December 23, 2003. The content of these proceeding applications are hereby incorporated by reference into this application.

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Throughout this application, various references are referred to. Disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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FIELD OF THE INVENTION

This invention relates to extracts from a plant called Wenguanguo (*Xanthoceras Sorbifolia*), their usages and functions, and methods of their preparation.

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BACKGROUND OF THE INVENTION

Wenguanguo is a species of the sapindaceae family. Its scientific name is *Xanthoceras sorbifolia* Bunge. Wenguanguo is the common Chinese name; others are Wenguannguo, Wenguanmu, Wenguanhua, Xilacedeng, Goldenhorn and Yellowhorn. Wenguanguo is grown in Liaoning, Jilin, Hebei, Shandong, Jiangsu, Henan, Shanxi, Shaanxi, Gansu, Ningxia and inner Mongolia, China. Its seeds, leaves and flowers are edible and have been used as a folk medicine to treat enuresis for centuries. Its branches and woods are also used as a folk medicine.

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Chinese patent applications CN 1092991A and CN 1092992A provided the methods for producing a medicine from Wenguanguo kernel powder for curing enuresis and enhancing cerebral functions. Chinese patent CN 1052636C provided a method for

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producing a medicine with ethanol extract from the Wenguanguo kernel powder for curing enuresis and enhancing cerebral functions. Journal of Shenyang University of Pharmacy (2001), 18(1), 53-56 disclosed the n-butanol extract from the wood of Wenguanguo, which had anti-inflammatory effect.

United States Patent Application Publication No. 20030096030 provided the extracts from the husks of Wenguanguo which are Bunkankasaponin A. B. C. D and two sterols for preventing cerebral aging, improving cerebral functions and curing enuresis, frequent micturition, urinary incontinence, dementia, weak intelligence, and increasing the body's ability to resist the activity of glycosuria.

United States Patent Application Publication No. 20030082293 disclosed the extracts Bunkankasaponin A. B. C. D, crude fats, crude protein and sugars from the shell of Wenguanguo.

U.S. Patent Number 6,616,943, issued on September 9, 2003, provides the composition comprising Wenguanguo combined extracts and the methods for preparing them and uses thereof. The methods for preparing the combined extract from the husks comprise the following steps: extracting Wenguanguo husks with an organic solvent (e.g. ethanol) to form an organic (e.g. ethanol) extract; removing the organic solvent (e.g. ethanol) from the organic (e.g. ethanol) extract to form aqueous extracts; and drying and sterilizing the aqueous extracts to form the combined extracts. The combined extracts contain saponins, saccharides, proteins and others. The extracts can be used for producing medicines or health foods for preventing cerebral aging, improving memory, improving cerebral functions and curing enuresis, frequent micturition, urinary incontinence, dementia, weak intelligence and Alzheimer's disease, autism, brain trauma, Parkinson's disease and other diseases caused by cerebral dysfunction. The medicines or health foods further comprise Vitamin B, Vitamin D, K, anti-oxidant, Cordyceps or

its extracts, ginkgo or its extracts, Echinacea, or its extracts, Huperzine A, folic acid, amino acids, creatine, fiber supplement or a combination thereof.

- 5 Yingjie Chen, Tadahiyo Takeda and Yukio Ogihara in Chem. Pharm. Bull 33(4)1387-1394(1985) describe a study on the constituent of *Xanthoceras sorbifolia* Bunge. See Section V. Saponins from the Fruits of *Xanthoceras sorbifolia*. Four new saponins were isolated from the fruits of *Xanthoceras sorbifolia* Bunge. The
10 structures of these saponins are bunkankasaponins A, B, C and D:

- (A) 22-O-acetyl-21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyl-3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)-
15 β -D-glucuronopyranosyl]protoaecigenin
(B) 22-O-acetyl-21-O-(3,4-di-O-angeloyl)- β -D-fucopyranosyl-3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)-
 β -D-glucuronopyranosyl]protoaecigenin
(C) 28-O-acetyl-21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyl-3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)-
20 β -D-glucuronopyranosyl]protoaecigenin
(D) 28-O-acetyl-21-O-(3,4-di-O-angeloyl)- β -D-fucopyranosyl-3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)-
 β -D-glucuronopyranosyl]protoaecigenin

- 25 Yingjie Chen, Tadahiyo Takeda and Yukio Ogihara in Chem. Pharm. Bull 33(3)1043-1048(1985) describe studies on the constituent of *Xanthoceras sorbifolia* Bunge. See Section IV. Structures of the Miner Prosapogenin. The prosapogenins from the partial
30 hydrilyzate of fruit saponin of *Xanthoceras sorbifolia* were examined, and are characterized as:

- 16-O-acetyl-21-O-(3,4-di-O-angeloyl- β -D-fucopyranosyl)protoaecigenin
35 22-O-acetyl-21-O-(3,4-di-O-angeloyl- β -D-fucopyranosyl)

protoaescigenin 3-O- β -D-glucuronopyranoside.

Yingjie Chen, Tadahiro Takeda and Yukio Ogihara in Chem. Pharm. Bull 33 (1) 127-134 (1985) describe studies on the constituent of
5 Xanthoceras sorbifolia Bunge. See Section III. Minor Prosapogenins aponins from the Fruits of Xanthoceras sorbifolia Bunge. The structure of 3 minor prosapogenins, obtained by acid hydrolysis of the crude saponin faction, were characterized as:

- 10 21-O-(3,4-di-O-angeloyl)- β -D-fucopyranosyltheasapogenol B,
21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyltheasapogenol B,
15 21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyl-22-O-acetylprotoaescigenin

Yingjie Chen, Tadahiro Takeda and Yukio Ogihara in Chem. Pharm. Bull 33 (4) 1387-1394 (1985) described a study on the constituent
20 of Xanthoceras sorbifolia Bunge. See Section II. Major Sapogenol and prosapogenin from the Fruits of Xanthoceras sorbifolia.

Cancer cells are defined by two heritable properties: (1) they
25 reproduce in defiance of normal restraints on cell division; and (2) they invade and colonize territories normally reserved for other cells.

Cancers require mutations of many genes to develop, and they
30 are classified according to the tissue and cell type from which they arise. Cancers arising from epithelial cells are named carcinomas; those arising from connective tissue or muscle cells are named sarcomas. In addition, there are cancers called leukemias, which are derived from hemopoietic cells; and
35 cancers derived from cells of the nervous system.

Cancers originating from different types of cells are, in general, very different diseases. Each cancer has characteristics that reflect its origin. Even when a cancer has metastasized and proliferated out of control, its origins can be traced back to a single, primary tumor. Therefore it is important to develop drugs against target cells with a specified character.

Ovarian cancer is the 5th leading cause of cancer death in women and the leading cause of death from gynecologic. In the United States, females have a 1.4 to 2.5% (1 out of 40-60 women) lifelong chance of developing ovarian cancer. Older women are at highest risk. More than half of the deaths from ovarian cancer occur in women between 55 and 74 years of age and approximately one quarter of ovarian cancer deaths occur in women between 35 and 54 years of age. (See <http://www.nlm.nih.gov/medlineplus/ency/article/000889.htm>)

Ovarian cancer is disproportionately deadly for a number of reasons. First, symptoms are vague and non-specific, so women and their physicians frequently attribute them to more common conditions. By the time the cancer is diagnosed, the tumor has often spread beyond the ovaries. Also, ovarian cancers shed malignant cells that frequently implant on the uterus, bladder, bowel, and lining of the bowel wall (omentum). These cells can begin forming new tumor growths before cancer is even suspected. Second, because no cost-effective screening test for ovarian cancer exists, more than 50 percent of women with ovarian cancer are diagnosed in the advanced stages of the disease.

This invention provides compounds or compositions extracted from *Xanthoceras Sorbifolia* or synthesized which have substantial potency against ovarian cancer.

SUMMARY OF THE INVENTION

In accordance with these and other objects of the invention, a brief summary of the present invention is presented. Some
5 simplifications and omission may be made in the following summary, which is intended to highlight and introduce some aspects of the present invention, but not to limit its scope. Detailed descriptions of a preferred exemplary embodiment adequate to allow those of ordinary skill in the art to make
10 and use the invention concepts will follow in later sections.

This invention is related to the use of extracts of Wenguanguo. The extracts of Wenguanguo can prevent patients from enuresis and frequency micturition. The extracts of Wenguanguo prevent
15 enuresis because it improves patients' cerebral functions.

This invention provides a composition to improve the functions of the central nervous system to help patients be more aware of the signal sent from the bladder and to wake themselves from
20 deep sleep.

This invention provides a composition which relaxes the bladder so that it can store more urine.

25 The extracts of Wenguanguo may be used to relax the detrusor tension caused by aging, stress, nervousness, over-activity, instability, hyper-reflexia, and uninhibited bladder. In another embodiment, the extracts of Wenguanguo may be used for relaxing the contracted bladder tissue induced by acetylcholine,
30 ACh.

The extracts of Wenguanguo may be used as acetylcholinesterase, AChE inhibitor, for regulating Antidiuretic hormone (ADH), which reduces the volume of urine and as an anti-inflammatory
35 agent.

Flavone may be extracted from the leaf, fruit-stem, branch, stem, root, and bark of the Wenguanguo and methods of their preparation. In an embodiment, this invention provides a composition comprising the flavone extracts from leaf, fruit-stem and branch or stem, root and bark which are flavonols, flavanols, dihydroflavonols, phenoloids, organic acids and others.

Extract from husks, leaves, branch or stem, and fruit-stem, root and bark of the Wenguanguo may be combined and this invention discloses method of their preparation.

This invention provides a composition comprising the combined extracts from the husks, leaf or branch, stem and or fruit-stem, root, seed's shell and bark of the Wenguanguo. The organic extracts contain saponins, saccharides, proteins, glycosides, flavonoids and others.

This invention provides the crude saponins from the husks, leaf or branch, stem and or fruit-stem root, seed's shell and bark of Wenguanguo and methods of their preparation. This invention provides a composition comprising the crude saponins from the husks, leave or branch, stem and or fruit-stem root and bark of the Wenguanguo. The crude saponins contain saponins, saccharides, proteins, and others.

This invention provides a process of producing curmarin extracts from the husk, kernel, leaf, fruit-stem, and branch, root, seed's shell and bark of Wenguanguo and their applications. This invention provides a composition comprising the curmarin extracts containing curmarins from the husks, kernel, leaf or branch, stem and or fruit-stem, root, seed's shell and bark of the Wenguanguo. The kurmarin extracts contain kurmarins, coumaric glycosides, saccharides, proteins, and others.

This invention provides a process of producing an aqueous

extract from the husk, kernel, leaf, fruit-stem, branch, stem, root, seed's shell and bark of Wenguanguo and their applications. This invention provides a composition comprising the aqueous extract from the husk, kernel, leaf, fruit-stem, branch, stem,
5 root, seed's shell and bark of Wenguanguo. The aqueous extract contains sugars, polysaccharides, glucosides, saponins, proteins, tannins and others.

This invention provides a process of producing alkaloid
10 extracts from the husk, kernel, leaf, fruit-stem, branch, stem, root, seed's shell and bark of Wenguanguo and their applications. This invention provides a composition comprising the alkaloid extracts from the husk, kernel, leaf, fruit-stem, branch, stem, root, seed's shell and bark of Wenguanguo. The alkaloid extracts
15 comprises alkaloids, coumarins, saccharides, proteins and others.

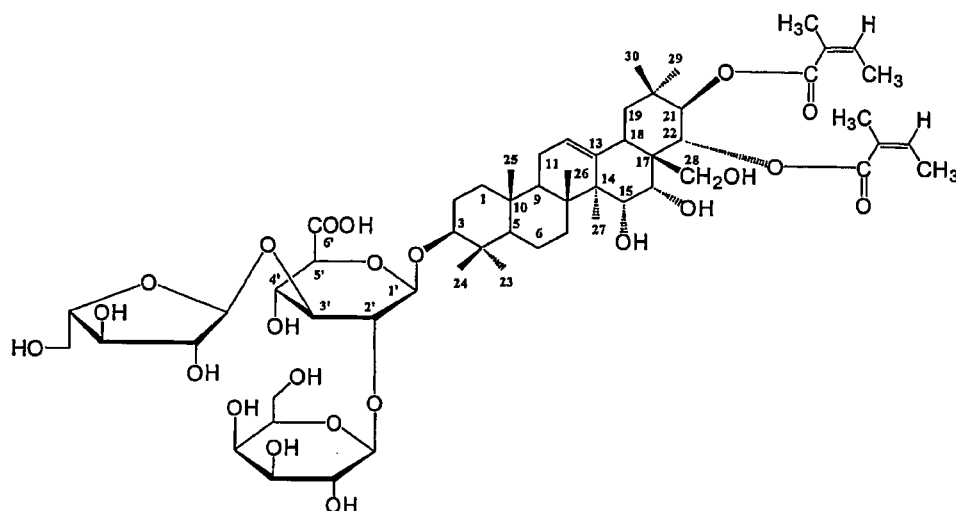
This invention provides a process of producing extract
comprises organic acids from husk, kernel, leaf, fruit-stem,
20 branch, stem, root, seed's shell and bark and their applications. This invention provides a composition comprising the extract containing crude organic acids from the husk, kernel, leaf, fruit-stem, branch, stem, root, seed's shell and bark of Wenguanguo. The extract comprises aromatic organic acids,
25 fatty organic acids, terpenoid organic acids, saccharides, proteins and others.

This invention provides two methods of producing tannin
extracts from Wenguanguo husk, branch, fruit-stem and leaf,
30 root, seed's shell and bark and its usages. 1) The method-1 for preparing the extracts with 95% ethanol. 2) The method-2 for preparing the extracts with acetone-water. This invention provides a composition comprising the tannin extracts from the husk, kernel, leaf, fruit-stem, and branch, root, seed's shell
35 and bark of Wenguanguo. The tannin extracts comprise tannins, organic acids, saccharides, proteins and others.

Wenguanguo extracts may be used for accelerating the growth of bladder, for suppressing deep sleep, for increasing alertness in a sleeping subject, for modulating the release, breakdown
5 and uptake of Antidiuretic hormone (ADH) and its receptors, for modulating the secretion, breakdown and uptake of Adrenocorticotrophic hormone (ACTH) and its receptors, for modulating the release, breakdown and uptake of
10 5-hydroxytryptamine and its receptors, for modulating the release, breakdown and uptake of Acetylcholine (Ach) and its receptors, for modulating the release, breakdown and uptake of Adrenaline (AD) and its receptors, for modulating the release, breakdown and uptake of Dopamine (DA) and its receptors, for
15 modulating the release, breakdown and uptake of Norepinephrine (NE) and its receptors, for preventing sleep paralysis, for modulating the formation, release, breakdown and activity of neuropeptides and their receptors, for curing cancer, including but not limited to breast cancer, leukocyte cancer, liver cancer, ovary cancer, bladder cancer, prostate cancer and brain cancer,
20 and for improving the functions of the lung and the bladder.

This invention provides a compound comprising a sugar, a terpene or Sapogenin, and a side chain at Carbon 21 and 22 or Angeloyl groups, operatively linked to form a biologically
25 active compound. In an embodiment, the compound comprises two or more sugars.

This invention provides a compound comprising the following structure:



This invention provides a salt of the above-described compounds.

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This invention provides a composition comprising the above-described compounds and a suitable carrier.

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This invention provides a pharmaceutical composition comprising an effective amount of the above-described compounds and a pharmaceutically acceptable carrier.

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This invention provides a method for isolating compounds from *Xanthoceras Sorbifolia* comprising steps of: extracting *Xanthoceras Sorbifolia* powder with an appropriate amount of one or more organic solvent for an appropriate amount of time to form an organic extract; collecting the organic extract; refluxing the organic extract with an organic solvent for an appropriate amount of time to form a second extract; removing the organic solvent from the second extract; and drying and sterilizing the second extract to form a *Xanthoceras Sorbifolia* extract powder.

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This invention provides a triterpenoid saponins with three sugars and one or more biangeloyl groups attached to the backbond.

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This invention provides a triterpenoid saponins with two sugars and one or more biangeloyl groups attached to the backbond.

5 This invention provides a triterpenoid saponins with four sugars and one or more biangeloyl groups attached to the backbond.

10 This invention provides a triterpenoid saponins with three sugars and one angeloyl group attached to the backbond.

This invention provides a method for isolating compounds from Xanthoceras Sorbifolia comprising steps of: extracting Xanthoceras Sorbifolia powder of with an appropriate amount of
15 one or more organic solvent for an appropriate amount of time to form an organic extract; collecting the organic extract; refluxing the organic extract to form a second extract; removing the organic solvent from the second extract; drying and sterilizing the second extract to form a Xanthoceras Sorbifolia
20 extract powder; fractionating the extract powder to obtain one or more components of the extract powder; identifying the bioactive components of the extract powder; purifying one or more bioactive components of the extract powder with FPLC to obtain one or more fraction of the bioactive component; and
25 isolating the compound with preparative HPLC.

This invention provides a compound having a NMR spectral data on the Heteronuclear Multiple Quantum Correlation (HMQC) study as shown in Table 2.

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This invention provides a compound having a NMR spectral data on Heteronuclear Multiple Bond Correlation (HMBC) study as shown in Table 3.

35 In accordance with these and other objects of the invention, a brief summary of the present invention is presented. Some

simplifications and omission may be made in the following summary, which is intended to highlight and introduce some aspects of the present invention, but not to limit its scope. Detailed descriptions of a preferred exemplary embodiment
5 adequate to allow those of ordinary skill in the art to make and use the invention concepts will follow in later sections.

This invention provides the pathways of a compound having the formula C57H88O23 and the chemical name
10 3-O- $[\beta$ -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene, and derivative compounds which are effective against cancer. The compounds or compositions of the present invention regulate the receptors
15 or components of cell such as G-protein receptor, Fas protein, receptor Tyrosine Kinases, Mitogen, mitogen receptor. The compounds can be isolated from the plant called Xanthoceras Sorbifolia or can be synthesized chemically, or extracted from other biological sources.

20 A normal stem cell division generates daughter cell with limited proliferation capacity. Daughter cell division generates non-dividing cells that is condemned to terminal differentiation and a cessation of cell division. However, if
25 a stem cell fails to produce a non-stem daughter in each division, it will proliferate to form a tumor. Also, if the daughter cell fails to differentiate normally, these cells will also proliferate to form a tumor.

30 Normal cells cease to divide when they mature into terminally differentiated, specialized cells. Once cells progress through a certain number of population doubling, they stop proliferating, a process termed replicative cell senescence. However, if a cell persists in dividing so as to generate a
35 cancer, it escapes the restraints of replicative senescence and avoids differentiation. These cells are genetically unstable,

and disregard the external and internal signals that regulate cell proliferation. They escape from their home tissues, and are therefore invasive. They can survive and proliferate in foreign sites, thereby causing more tumors.

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Abnormal changes in components' activities in pathways cause the cells to fail to stop proliferating so as to form cancer. The pathways include TGF Beta-smad, FGF, TGF-beta and TGF-alphaa, ras-GTPase-MAP kinase, jun-fos, Src-fyn, 10 Jak-Jnk-STAT, BMP, Wnt, myc-cell proliferation, etc. The mutation of cancer cell causes the cell-death program to become inactive, allowing cells to divide indefinitely. The Xanthoceras Sorbifolia derived compound and/or composition regulates the components and receptors and re-activates the 15 cell death program.

DETAILED DESCRIPTION OF THE FIGURES

Figure 1(a) and (b). The water maze learning effect of plant extract administration of aging mice for 9 days.

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Figure 2(a) and (b). The result of water maze learning of 3 days injected pentobarbital.

Figure 3. Effects of the extract X and Y on the quantity of urine in mice after 10 days of administration of X and Y.

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Figure 4. Shows the sleep cycle of a typical person.

Figure 5. Shows the HPLC Profile of Wanguanguo husk extract.

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Figure 6. Shows the efficacy of Wanguanguo extracts in inhibiting breast cancer cells, where IC₅₀ is 65µg/ml and IC₀ is 105µg/ml. Experimental results in Figure 6 also showed that the extracts inhibit leukocyte cancer cells, where IC₅₀ is 35µg/ml and IC₀ is 50µg/ml.

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Figure 7. Shows the efficacy of the Wanguanguo extracts in increasing the activity of bladder cells. The bladder cells grow up to 125% in extract concentration of 10µg/ml. Experimental results in Figure 7 also showed that wanguanguo extracts inhibit bladder cancer cells in high concentration extract with IC₅₀ - 45 and IC₀ - 60. Experimental results in Figure 7 showed that Wanguanguo extracts inhibit ovary cancer cells, where IC₅₀ is 15µg/ml and IC₀ is 20µg/ml.

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Figure 8. Shows the efficacy of Wanguanguo extracts in increasing the activity of brain cells. Brain cells grow up to 108% in extract concentration of 10µg/ml. Experimental results in Figure 8 showed that the extracts

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inhibit prostate cancer cells, where IC₅₀ is 40µg/ml and IC₀ is 70µg/ml. Experimental results in Figures 8 shows that the extracts inhibit brain cancer cells, where IC₅₀ is 70µg/ml and IC₀ is 100µg/ml.

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Figure 9. Shows the efficacy of the Wanguanguo extracts in increasing the activity of lung cells. Experimental results in Figure 9 showed that lung cells began to grow in culture medium RPMI-1640 containing extract concentration of 10ug/ml. Experimental results in Figure 9 also showed that lung cells grew up to 120% in extract concentration of 50ug/ml. Figure 9 also showed the efficacy of Wanguanguo extract in increasing the activity of bladder cells. The bladder cells grow up to 125% in culture medium RPMI-1640 containing extract concentration of 10µg/ml. Experimental results in Figure 9 also showed that the extracts inhibit bladder cancer cells in high concentration extract with IC₅₀ - 45 and IC₀ - 60. Experimental results in Figure 9 also showed that the extracts inhibit liver cancer cells, where IC₅₀ is 68µg/ml and IC₀ is 95µg/ml.

Figure 10. Shows the Wanguanguo extracts has slight effect on cervix cells.

Figure 11. Shows the efficacy of Wanguanguo extract in increasing the activity of bone cells. The bone cells grow up to 120% in culture medium RPMI-1640 containing extract concentration of 10µg/ml.

Experimental results in Figure 11 also showed that the extracts inhibit bone cancer cells in high concentration extract with IC₅₀ - 40 and IC₀ - 50.

Figure 21. Shows the separation of an extract of Xanthoceras Sorbifolia components by HPLC with a µbondapak C18 column.

- Figure 21A-M.** Shows the isolation of Fraction Y From an extract of *Xanthoceras Sorbifolia* by FPLC. A and B are representative elution profile of FPLC chromatograms. C - K are HPLC profiles of fractions obtained from the FPLC chromatograms. L: original materials. M: TLC chromatography. Selected fractions from FPLC were analyzed with TLC stained with carbohydrates.
- Figure 22.** Shows the absorption spectrum of *Xanthoceras Sorbifolia* extract.
Abscissa: Wavelength in nm
Ordinate: Optical Density.
- The extract has three absorption maximum at 207nm, 278nm and 500nm.
- Figure 23A-23E.** Shows the growth curves of cells after treatment with the extract of *Xanthoceras Sorbifolia* as determined by MTT assay. These studies reported the sensitivities of different cell lines toward the extract of *Xanthoceras Sorbifolia*.
- (23A) Most sensitive: Ovary.
(23B) Sensitive: bladder and bone.
(23C) Semi-sensitive: leukocyte and liver.
(23D) Marginal sensitive: prostate, breast and brain.
(23E) Least sensitive: colon, cervix and lung.
- Figure 24.** Shows the elution profile of an extract of *Xanthoceras Sorbifolia* in FPLC with 10-80% gradient.
Ordinate: optical density.
Abscissa: fraction volume (5ml/fraction).
- Figure 25.** Shows screening of cell growth activity of FPLC fractions (conducted with bladder cells). 25A Shows the

elution fractions obtained from FPLC. These fractions were subsequently used in MTT assay to determine which fraction is active. 25B shows that different components of *Xanthoceras Sorbifolia* extracts (as fractionated by FPLC) cause either growth or inhibition effects on cells. Only fraction 5962 (Y components) cause cell inhibition. Fractions 610 and 1116 cause minor stimulation of cell growth.

10 Abscissa: concentration (ul/ml)
Ordinate: % Cell Growth (determined by MTT assay).

Figure 26. Shows elution profile of Fraction 5962 with 64% isocratic elution. Two major FPLC fractions X and Y are separated.)

Ordinate: optical density.

Abcissa: fraction number (1ml/fraction).

Figure 27. Shows the comparison of inhibition activity in bladder cells by Fractions X (2021) and Y (2728). Only Y has inhibition activity.

Figure 28. Shows the HPLC profile of Fraction Y with 35% isocratic elution. Figure 28 also shows on 35% isocratic analysis, the Y (2728) fraction contains 4-5 components (Y0, Y1, Y2, Y3 and Y4).

Figure 29. Shows HPLC profile of Fraction Y with 45% Acetonitrile isocratic elution. Figure 29 also shows on 45% isocratic analysis, Y3 and Y4 peaks merge into one peak. In this condition, the fractions Y1 and 2 are nicely separated from fractions Y3 and Y4.

Figure 30. Shows HPLC profile of Fraction Y with 45% Acetonitrile isocratic elution in a preparative C18 column (Delta Pak C18). Under these conditions, fractions Y1 and

Y2 are well separated from each other and they are collected individually.

5 **Figure 31.** Compares Proton-NMR profiles of ascending (upper curve) and descending (lower curve) portion of peak Y(3/4) and shows they are identical.

Figure 32. Shows HPLC profile of collected Fraction Y with 45% isocratic elution in a preparative C18 column.

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Figure 33. Shows Potency of purified Fraction Y on ovarian cancer cells (OCAR-3).

15 Abscissa: concentration (ug/ml). **A:** Point scale. **B** Linear scale.

Ordinate: % Cell Growth (determined by MTT). The IC50 is approximately 1 ug/ml.

20 **Figure 34.** Shows comparison of potency of Fraction Y toward ovarian cancer cells and cervical cancer cells. Ovarian cancer cells are sensitive to Fraction Y while cervical cancer cells are not. The IC50 for Fraction Y in ovary cells is about 1. This result confirms that ovary cancer is sensitive to purified Fraction Y.

25

Figure 35. Shows the chemical shift of the proton NMR of Fraction Y.

30 **Figure 36.** Shows HMQC results of Sample Y. Also see Table 22 for listed chemical shift data.

Figure 37. Shows HMBC results of Y. **A:** level one. **B:** level 2 Also see Table 23 for listed chemical shift data.

35 **Figure 38.** Shows the Mass spectroscopy of Y compound. (A) MALDI-TOF (high mass) **A1:** Y + Matrix (CHCA) + Angiotensin 1

"two point calibration". A2: Y + Matrix (CHCA) + Angiotensin "one point calibration". A3: Y + Matrix (CHCA). A4: Matrix (CHCA) only. A5: Angiotensin 1 + Matrix (CHCA). (B) ESI-MS.

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Figure 39. Shows the structure and chemical name of Y:
3-O- $[\beta$ -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene.

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Figure 40. Shows the Proton NMR of Y1. Chemical shift data is presented in Table 24.

Figure 41. Shows 2D NMR of Y1 (HMQC) with chemical shift data (table 25).

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Figure 42. Shows the proton NMR of Y2 with chemical shift data (Table 26).

Figure 43. Shows 2D NMR of Y2 (HMQC) with chemical shift data (Table 27).

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Figure 44. Shows proton NMR of Y4 with chemical shift data (Table 28).

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Figure 45. Shows 2D NMR of Y4 (HMQC) with chemical shift data (Table 29).

Figure 46. Shows COSY-NMR profile of Y1 with chemical shift data (Table 30).

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Figure 47. Shows three possible Structures of Y1. A: structure Y1-1; B: structure Y1-2; and C: structure Y1-3.

Table24.txt

DU=C:/Bruker/XWIN-NMR, USER=guest, NAME=chan, EXPNO=8, PROCNO=1
 F1=10.000ppm, F2=0.000ppm, MI=0.05cm, MAXI=10000.00cm, PC=1.000

#	ADDRESS	FREQUENCY [Hz]	INTENSITY [PPM]
1	5803.6	5312.007	1.05
2	6201.8	5224.701	180.00
3	6517.2	5155.558	0.09
4	6612.7	5134.626	1.05
5	8992.9	4612.799	0.41
6	9356.8	4533.029	62.60
7	9733.1	4450.514	0.40
8	9952.7	4402.383	0.13
9	9996.2	4392.832	0.90
10	10361.5	4312.746	134.51
11	10641.7	4251.327	0.05
12	10739.1	4229.980	0.87
13	12483.6	3847.521	0.10
14	12504.0	3843.034	0.10
15	13117.1	3708.630	0.31
16	13161.7	3698.843	0.31
17	13503.5	3623.905	0.38
18	13617.2	3598.988	0.08
19	13649.9	3591.811	0.19
20	13679.2	3585.404	0.18
21	13712.9	3577.998	0.08
22	13886.8	3539.884	0.09
23	13920.0	3532.594	0.19
24	13952.5	3525.479	0.17
25	13983.6	3518.658	0.06
26	14413.7	3424.364	0.36
27	14428.7	3421.080	0.36
28	14720.0	3357.206	0.19
29	14735.6	3353.801	0.18
30	14766.4	3347.042	0.20
31	14781.9	3343.652	0.17
32	15028.7	3289.533	0.05
33	15343.4	3220.543	0.33
34	15404.6	3207.131	0.25
35	15439.6	3199.461	0.25
36	16193.8	3034.111	0.14
37	16238.8	3024.240	0.15
38	16381.2	2993.014	0.17
39	16518.3	2962.967	0.12
40	16553.7	2955.209	0.12
41	16599.3	2945.198	0.08
42	16633.5	2937.700	0.08
43	16843.2	2891.736	0.18
44	16905.3	2878.117	0.17
45	16927.0	2873.370	0.19
46	17523.6	2742.566	0.33
47	17586.3	2728.818	0.18
48	17617.6	2721.965	0.23
49	17635.4	2718.066	0.31
50	17665.9	2711.371	0.32
51	17716.2	2700.349	0.21
52	17760.5	2690.632	0.20
53	17810.9	2679.588	0.55
54	17835.7	2674.141	0.72
55	17857.9	2669.267	0.54
56	18007.8	2636.409	0.17
57	18046.2	2627.986	0.26
58	18083.2	2619.886	0.19
59	18189.9	2596.493	0.11

Table24.txt			
60	18233.6	2586.912	4.3104 0.27
61	18277.8	2577.209	4.2942 0.31
62	18331.5	2565.443	4.2746 0.21
63	18367.6	2557.542	4.2614 0.23
64	18412.4	2547.721	4.2451 0.19
65	18458.8	2537.540	4.2281 0.10
66	18491.9	2530.283	4.2160 0.21
67	18532.6	2521.359	4.2011 0.28
68	18567.2	2513.770	4.1885 0.14
69	18645.4	2496.625	4.1599 0.25
70	18666.5	2491.997	4.1522 0.25
71	18699.9	2484.686	4.1400 0.24
72	18721.5	2479.943	4.1321 0.19
73	18778.7	2467.413	4.1113 0.20
74	18792.8	2464.305	4.1061 0.20
75	18821.9	2457.939	4.0955 0.19
76	18835.7	2454.909	4.0904 0.18
77	19186.2	2378.063	3.9624 0.17
78	19213.7	2372.029	3.9523 0.29
79	19239.6	2366.350	3.9429 0.16
80	19338.7	2344.635	3.9067 0.08
81	19366.7	2338.490	3.8964 0.22
82	19396.9	2331.872	3.8854 0.21
83	19428.2	2325.007	3.8740 0.06
84	20015.2	2196.324	3.6596 0.05
85	20048.6	2188.994	3.6474 0.08
86	20131.1	2170.900	3.6172 0.13
87	20161.7	2164.211	3.6061 0.28
88	20208.1	2154.040	3.5891 0.31
89	20679.1	2050.765	3.4170 0.24
90	20727.6	2040.135	3.3993 0.23
91	20782.4	2028.119	3.3793 0.08
92	21179.2	1941.123	3.2343 0.14
93	21213.6	1933.579	3.2218 0.13
94	21586.4	1851.862	3.0856 0.07
95	21649.4	1838.049	3.0626 0.09
96	21715.8	1823.490	3.0383 0.67
97	21747.5	1816.527	3.0267 0.23
98	23153.9	1508.199	2.5130 0.06
99	23693.5	1389.916	2.3159 2.02
100	24231.4	1271.971	2.1194 0.22
101	24282.2	1260.831	2.1008 0.22
102	24341.3	1247.878	2.0792 0.16
103	24413.6	1232.024	2.0528 0.15
104	24464.0	1220.989	2.0344 0.83
105	24468.7	1219.963	2.0327 0.83
106	24496.7	1213.827	2.0225 0.82
107	24540.5	1204.212	2.0065 0.24
108	24614.2	1188.066	1.9796 0.24
109	24658.3	1178.391	1.9635 0.88
110	24686.0	1172.311	1.9533 0.86
111	24690.9	1171.249	1.9516 0.85
112	24774.5	1152.908	1.9210 1.65
113	24824.7	1141.905	1.9027 0.22
114	24966.5	1110.815	1.8509 2.07
115	25021.9	1098.679	1.8306 2.09
116	25154.1	1069.689	1.7823 0.22
117	25213.5	1056.676	1.7607 0.20
118	25280.4	1042.010	1.7362 0.33
119	25347.2	1027.363	1.7118 0.24
120	25396.2	1016.604	1.6939 0.38
121	25591.3	973.839	1.6226 0.16
122	25626.4	966.149	1.6098 0.22

Table24.txt

123	25673.7	955.785	1.5926	0.16
124	25759.1	937.061	1.5614	0.45
125	25837.0	919.968	1.5329	0.32
126	25932.6	899.023	1.4980	0.22
127	25995.9	885.130	1.4748	0.28
128	26142.6	852.967	1.4212	1.52
129	26284.2	821.936	1.3695	1.99
130	26355.3	806.354	1.3436	0.49
131	26405.2	795.414	1.3253	0.53
132	26437.0	788.423	1.3137	0.61
133	26495.4	775.620	1.2924	1.83
134	26547.6	764.186	1.2733	0.62
135	26578.6	757.400	1.2620	0.75
136	26635.0	745.018	1.2414	0.66
137	26710.2	728.529	1.2139	0.35
138	26742.2	721.515	1.2022	0.34
139	26774.1	714.532	1.1906	0.38
140	26818.0	704.909	1.1745	1.47
141	26852.6	697.329	1.1619	2.39
142	26939.0	678.382	1.1303	0.28
143	26967.1	672.226	1.1201	0.28
144	26987.0	667.851	1.1128	0.31
145	27035.8	657.144	1.0949	0.25
146	27059.9	651.863	1.0861	0.21
147	27091.3	644.992	1.0747	0.18
148	27116.3	639.514	1.0656	0.13
149	27306.1	597.891	0.9962	0.21
150	27331.8	592.271	0.9869	0.20
151	27362.1	585.622	0.9758	0.18
152	27426.7	571.457	0.9522	0.07
153	27465.2	563.025	0.9381	0.12
154	27550.1	544.395	0.9071	0.06
155	27604.4	532.507	0.8873	0.09
156	27723.7	506.347	0.8437	0.23
157	27778.4	494.349	0.8237	0.48
158	27825.7	483.975	0.8064	1.88
159	27892.5	469.335	0.7820	1.79
160	27999.1	445.968	0.7431	0.28
161	28054.1	433.917	0.7230	0.23
162	28134.1	416.375	0.6938	0.08
163	28228.5	395.678	0.6593	0.07
164	28262.5	388.209	0.6468	0.09

Table25.txt

DU=C:/Bruker/XWIN-NMR, USER=guest, NAME=chan, EXPNO=18, PROCNO=1
 F1PLO=116.085ppm, F1PHI=10.797ppm, F2PLO=6.538ppm, F2PHI=0.368ppm
 MI=1.00cm, MAXI=10000.00cm, PC=2.000

#	ADDRESS row col	FREQUENCY		INTENSITY
		[Hz] F1 [Hz] F2	[PPM] F1 [PPM] F2	
1	315	16740.193	110.9281	30.72
	375	3624.445	6.0391	
2	352	15855.943	105.0686	20.34
	490	2950.420	4.9161	
3	450	13537.575	89.7061	20.34
	490	2950.420	4.9161	
4	479	12863.844	85.2416	12.42
	476	3032.063	5.0521	
5	479	12863.844	85.2416	17.76
	500	2892.360	4.8193	
6	491	12577.806	83.3462	25.72
	487	2969.087	4.9472	
7	522	11857.070	78.5703	13.55
	545	2627.024	4.3772	
8	529	11685.553	77.4337	16.93
	504	2870.087	4.7822	
9	535	11537.104	76.4500	15.06
	589	2370.150	3.9492	
10	550	11191.633	74.1608	12.94
	421	3355.320	5.5907	
11	550	11191.633	74.1608	15.14
	423	3342.807	5.5699	
12	569	10733.316	71.1238	24.93
	410	3421.295	5.7006	
13	579	10497.793	69.5631	26.50
	526	2740.480	4.5662	
14	586	10332.951	68.4708	14.53
	537	2674.056	4.4556	
15	586	10332.951	68.4708	11.00
	596	2330.159	3.8826	
16	617	9621.364	63.7555	12.23
	643	2051.886	3.4189	
17	630	9299.908	61.6254	13.25
	529	2718.381	4.5294	
18	630	9299.908	61.6254	14.42
	539	2665.365	4.4411	
19	669	8391.979	55.6090	13.25
	529	2718.381	4.5294	

Table25.txt

20	669 539	8391.979 2665.365	55.6090 4.4411	14.42
21	720 682	7193.972 1821.991	47.6705 3.0358	10.83
22	725 820	7055.427 1015.280	46.7524 1.6917	8.84
23	770 682	5994.782 1822.241	39.7241 3.0363	21.78
24	778 682	5810.328 1822.241	38.5019 3.0363	21.78
25	803 836	5229.784 918.974	34.6549 1.5312	9.20
26	813 866	4978.483 743.550	32.9897 1.2389	9.04
27	833 847	4517.225 852.860	29.9332 1.4211	64.24
28	847 861	4185.850 773.417	27.7373 1.2887	74.68
29	850 806	4113.891 1096.582	27.2605 1.8271	80.21
30	850 861	4113.891 773.392	27.2605 1.2886	31.46
31	885 756	3291.884 1389.088	21.8135 2.3145	100.00
32	893 796	3110.641 1153.093	20.6125 1.9213	89.09
33	893 803	3110.641 1110.425	20.6125 1.8502	50.66
34	897 803	2998.505 1111.101	19.8694 1.8513	45.64
35	897 853	2998.505 820.195	19.8694 1.3666	78.07
36	919 874	2497.783 699.217	16.5514 1.1651	72.04
37	919 910	2497.783 483.491	16.5514 0.8056	37.18
38	926 794	2336.725 1165.059	15.4842 1.9412	17.70
39	926 910	2336.725 484.392	15.4842 0.8071	31.53

Table26.txt

DU=C:/Bruker/XWIN-NMR, USER=guest, NAME=chan, EXPNO=10, PROCNO=1
 F1=10.000ppm, F2=0.000ppm, MI=0.05cm, MAXI=10000.00cm, PC=1.000

#	ADDRESS	FREQUENCY [Hz]	INTENSITY [PPM]
1	5810.0	5312.575	1.10
2	6208.2	5225.282	180.00
3	6521.4	5156.623	0.08
4	6618.9	5135.231	1.06
5	8991.6	4615.051	0.48
6	9355.8	4535.208	72.14
7	9732.2	4452.690	0.47
8	9953.1	4404.253	0.12
9	9995.8	4394.912	1.07
10	10361.4	4314.740	154.22
11	10739.1	4231.938	1.01
12	11670.5	4027.748	0.27
13	11716.8	4017.596	0.28
14	12142.6	3924.242	0.09
15	12191.6	3913.512	0.08
16	12720.2	3797.615	0.29
17	12767.0	3787.357	0.27
18	13474.1	3632.344	0.38
19	13691.6	3584.643	0.18
20	13724.3	3577.480	0.17
21	13894.9	3540.074	0.08
22	13926.6	3533.142	0.07
23	14192.8	3474.762	0.15
24	14224.4	3467.833	0.15
25	14849.8	3330.724	0.10
26	15031.1	3290.991	0.56
27	15462.8	3196.344	0.10
28	15495.0	3189.283	0.07
29	16285.8	3015.906	0.20
30	16380.7	2995.102	0.35
31	16656.9	2934.562	0.12
32	16691.7	2926.933	0.09
33	16808.4	2901.328	0.20
34	16918.3	2877.252	0.23
35	17364.1	2779.505	0.09
36	17545.5	2739.737	0.32
37	17580.6	2732.048	0.35
38	17621.8	2723.010	0.33
39	17671.0	2712.235	0.22
40	17720.0	2701.484	0.25
41	17766.6	2691.267	0.25
42	17830.7	2677.212	0.49
43	17883.7	2665.595	0.54
44	18046.4	2629.914	0.15
45	18079.4	2622.681	0.17
46	18118.5	2614.108	0.12
47	18193.9	2597.588	0.49
48	18246.1	2586.136	0.56
49	18295.9	2575.231	0.51
50	18344.7	2564.538	0.44
51	18441.6	2543.273	0.40
52	18564.0	2516.452	0.41
53	18616.9	2504.857	0.29
54	18642.0	2499.356	0.32
55	18692.3	2488.315	0.81
56	18721.0	2482.035	1.28
57	18749.9	2475.688	0.66
58	18812.7	2461.931	0.12
59	18923.7	2437.591	0.14

			Table26.txt	
60	18959.7	2429.692	4.0484	0.21
61	18999.0	2421.074	4.0340	0.10
62	19242.9	2367.603	3.9450	0.12
63	19783.6	2249.061	3.7474	0.21
64	19832.2	2238.424	3.7297	0.23
65	20066.8	2186.983	3.6440	0.20
66	20108.3	2177.874	3.6288	0.22
67	20209.5	2155.707	3.5919	0.07
68	20452.9	2102.343	3.5030	0.22
69	20501.2	2091.755	3.4853	0.21
70	20699.2	2048.331	3.4130	0.16
71	20750.3	2037.137	3.3943	0.14
72	20777.5	2031.179	3.3844	0.10
73	20994.3	1983.637	3.3052	0.13
74	21033.2	1975.112	3.2910	0.13
75	21183.1	1942.257	3.2362	0.07
76	21464.1	1880.637	3.1336	0.61
77	21493.2	1874.264	3.1229	1.18
78	21522.4	1867.862	3.1123	0.63
79	21602.1	1850.400	3.0832	0.27
80	21648.8	1840.141	3.0661	0.63
81	21709.2	1826.899	3.0440	0.14
82	23167.5	1507.197	2.5113	0.05
83	23193.4	1501.524	2.5019	0.06
84	23228.5	1493.830	2.4891	0.05
85	23464.1	1442.173	2.4030	0.12
86	23673.4	1396.297	2.3265	0.10
87	23717.4	1386.638	2.3104	0.12
88	23813.9	1365.486	2.2752	0.14
89	23867.3	1353.767	2.2557	0.15
90	24076.0	1308.020	2.1795	0.06
91	24237.2	1272.687	2.1206	0.37
92	24315.0	1255.625	2.0922	0.94
93	24347.2	1248.573	2.0804	0.87
94	24412.7	1234.206	2.0565	0.43
95	24444.9	1227.149	2.0447	0.44
96	24477.4	1220.016	2.0328	0.35
97	24546.3	1204.918	2.0077	1.65
98	24630.5	1186.456	1.9769	0.83
99	24667.0	1178.457	1.9636	0.98
100	24698.9	1171.464	1.9519	0.94
101	24776.1	1154.543	1.9237	0.24
102	24829.9	1142.750	1.9041	0.47
103	24905.2	1126.225	1.8765	0.36
104	25001.0	1105.220	1.8415	2.25
105	25283.4	1043.323	1.7384	1.76
106	25374.5	1023.343	1.7051	0.44
107	25400.9	1017.554	1.6955	0.44
108	25497.2	996.442	1.6603	0.18
109	25543.0	986.402	1.6436	0.30
110	25569.3	980.631	1.6340	0.30
111	25684.4	955.412	1.5919	0.33
112	25758.5	939.160	1.5648	0.50
113	25933.0	900.905	1.5011	0.13
114	26000.7	886.055	1.4764	0.17
115	26171.3	848.668	1.4141	0.59
116	26211.0	839.946	1.3995	0.50
117	26300.6	820.314	1.3668	0.36
118	26336.4	812.459	1.3537	0.33
119	26431.5	791.614	1.3190	2.36
120	26483.1	780.304	1.3002	2.02
121	26582.9	758.422	1.2637	0.85
122	26636.1	746.759	1.2443	0.53

			Table26.txt	
123	26687.2	735.552	1.2256	0.39
124	26747.9	722.238	1.2034	0.27
125	26849.0	700.084	1.1665	0.61
126	26891.3	690.817	1.1511	0.87
127	26942.7	679.537	1.1323	0.75
128	26970.8	673.382	1.1220	0.64
129	27001.4	666.680	1.1108	0.79
130	27042.5	657.660	1.0958	1.81
131	27092.2	646.759	1.0776	0.57
132	27315.3	597.860	0.9962	0.29
133	27367.3	586.458	0.9772	0.16
134	27392.2	580.997	0.9681	0.17
135	27468.0	564.369	0.9404	1.46
136	27583.9	538.958	0.8980	0.30
137	27639.5	526.783	0.8777	0.31
138	27696.4	514.300	0.8569	0.23
139	27755.5	501.356	0.8354	0.72
140	27809.8	489.450	0.8155	0.51
141	27898.0	470.100	0.7833	0.39
142	27949.8	458.759	0.7644	0.23
143	28010.2	445.501	0.7423	0.23
144	28179.5	408.388	0.6805	0.09
145	28270.7	388.387	0.6471	1.43
146	29248.3	174.074	0.2900	0.33
147	29267.3	169.910	0.2831	0.57
148	29290.2	164.891	0.2747	0.30

Assignment		w1	Table27.txt w2 Data Height
1C-H	111.010	6.048	18826190
2C-H	104.414	4.887	14324975
3C-H	103.805	5.490	9309093
4C-H	86.164	4.245	7318077
5C-H	85.321	4.832	11463916
6C-H	83.485	4.980	13272370
7C-H	82.312	1.199	6422175
8C-H	78.364	4.297	7384251
9C-H	78.367	4.216	7043049
10C-H	77.957	4.239	6649256
11C-H	77.264	4.786	12573529
12C-H	75.451	4.045	7102450
13C-H	75.251	4.029	6339352
14C-H	73.228	6.323	8991836
15C-H	73.305	4.426	16257361
16C-H	72.991	6.302	9674724
17C-H	72.491	4.457	9585418
18C-H	69.293	4.561	10388180
19C-H	67.362	4.192	13801715
20C-H	64.934	4.334	9238968
21C-H	63.243	3.727	6145056
22C-H	62.935	3.477	6603994
23C-H	62.608	4.290	11417088
24C-H	62.099	4.309	14518441
25C-H	61.917	4.329	12294503
26C-H	61.618	4.456	11029088
27C-H	61.271	4.519	7515041
28C-H	60.762	4.130	39131184
29C-H	47.047	1.417	7894303
30C-H	42.600	3.119	34316564
31C-H	40.719	3.066	12374124
32C-H	29.598	1.127	20143540
33C-H	29.310	1.094	58191532
34C-H	28.540	1.291	8016292
35C-H	27.916	1.263	19194828
36C-H	27.396	1.832	8506404
37C-H	21.984	1.296	49758680
38C-H	20.800	2.003	46698880
39C-H	20.414	1.738	53046288
40C-H	19.973	1.315	70912128
41C-H	18.664	1.117	9230194
42C-H	18.654	1.087	8675350
43C-H	17.509	0.991	9315817
44C-H	17.143	0.947	25886530
45C-H	16.953	1.156	19760612
46C-H	15.534	2.104	10787224
47C-H	15.642	2.069	13369578
48C-H	15.572	0.671	16296111
49C-H	15.523	0.643	7321235
50C-H	15.403	1.986	9376028
51C-H	15.484	1.971	11124703
52C-H	15.462	1.931	8888843
53C-H	15.369	0.848	9251849
54C-H	15.501	0.817	10315735
55C-H	15.472	0.621	13161576
56C-H	125.103	5.484	12492876
57C-H	137.121	5.955	6751365
58C-H	136.141	5.784	6797706
59C-H	136.290	2.613	6207858
60C-H	77.877	4.270	7046906
61C-H	20.915	1.839	56088448

62C-H	20.739	1.976	14307166
63C-H	17.164	0.932	17254234
64C-H	1.101	0.314	7575608

Table27.txt

Table28.txt

DU=C:/Bruker/XWIN-NMR, USER=guest, NAME=chan, EXPNO=12, PROCNO=1
 F1=10.000ppm, F2=0.000ppm, M1=0.05cm, MAXI=10000.00cm, PC=1.000

#	ADDRESS	FREQUENCY [Hz]	INTENSITY [PPM]
1	5893.1	5312.116	8.8512
2	6291.4	5224.804	8.7057
3	6594.0	5158.458	8.5951
4	6702.5	5134.662	8.5555
5	9068.0	4616.059	7.6914
6	9431.9	4536.298	7.5585
7	9807.9	4453.850	7.4211
8	10072.9	4395.768	7.3243
9	10438.5	4315.603	7.1908
10	10816.0	4232.842	7.0529
11	11735.2	4031.320	6.7171
12	11783.3	4020.770	6.6995
13	11907.4	3993.561	6.6542
14	11953.6	3983.450	6.6373
15	12000.0	3973.260	6.6203
16	12045.6	3963.270	6.6037
17	12788.6	3800.379	6.3323
18	12837.0	3789.776	6.3146
19	13063.5	3740.104	6.2318
20	13110.2	3729.878	6.2148
21	13430.2	3659.725	6.0979
22	13511.5	3641.891	6.0682
23	13544.5	3634.661	6.0562
24	13586.3	3625.502	6.0409
25	13773.4	3584.471	5.9725
26	13805.1	3577.516	5.9609
27	13959.1	3543.757	5.9047
28	13991.4	3536.681	5.8929
29	14274.1	3474.695	5.7896
30	14308.1	3467.252	5.7772
31	14488.2	3427.770	5.7114
32	14940.9	3328.527	5.5461
33	15103.6	3292.839	5.4866
34	15159.3	3280.638	5.4663
35	15507.7	3204.257	5.3390
36	15544.4	3196.217	5.3256
37	15767.1	3147.391	5.2443
38	15801.5	3139.847	5.2317
39	16228.5	3046.228	5.0757
40	16379.8	3013.070	5.0204
41	16567.9	2971.822	4.9517
42	16675.3	2948.287	4.9125
43	16709.1	2940.870	4.9001
44	16911.3	2896.548	4.8263
45	16994.9	2878.213	4.7957
46	17015.0	2873.803	4.7884
47	17141.0	2846.176	4.7424
48	17169.3	2839.983	4.7320
49	17250.9	2822.087	4.7022
50	17471.4	2773.741	4.6217
51	17511.3	2764.997	4.6071
52	17545.5	2757.491	4.5946
53	17627.9	2739.433	4.5645
54	17705.3	2722.464	4.5362
55	17738.6	2715.167	4.5241
56	17779.9	2706.102	4.5090
57	17841.2	2692.678	4.4866
58	17904.3	2678.844	4.4636
59	17946.6	2669.557	4.4481

			Table28.txt	
60	17977.6	2662.764	4.4368	0.79
61	18052.7	2646.299	4.4093	0.75
62	18074.7	2641.481	4.4013	0.79
63	18117.9	2632.004	4.3855	0.77
64	18157.7	2623.291	4.3710	0.55
65	18195.2	2615.072	4.3573	0.36
66	18282.0	2596.030	4.3256	0.27
67	18317.3	2588.301	4.3127	0.65
68	18370.2	2576.697	4.2934	0.70
69	18420.2	2565.727	4.2751	0.25
70	18465.5	2555.811	4.2585	0.30
71	18522.0	2543.424	4.2379	0.33
72	18578.7	2530.989	4.2172	0.73
73	18616.7	2522.647	4.2033	0.99
74	18651.8	2514.951	4.1905	0.49
75	18726.0	2498.696	4.1634	0.52
76	18745.7	2494.373	4.1562	0.53
77	18784.8	2485.790	4.1419	0.47
78	18800.9	2482.260	4.1360	0.44
79	18889.5	2462.838	4.1036	0.44
80	18926.1	2454.821	4.0903	0.38
81	18967.8	2445.677	4.0750	0.17
82	19005.7	2437.367	4.0612	0.19
83	19038.6	2430.166	4.0492	0.09
84	19310.2	2370.607	3.9500	0.58
85	19335.4	2365.098	3.9408	0.35
86	19850.6	2252.132	3.7526	0.46
87	19898.8	2241.578	3.7350	0.41
88	20201.0	2175.321	3.6246	0.07
89	20292.2	2155.333	3.5913	0.05
90	20490.7	2111.804	3.5187	0.08
91	20538.0	2101.428	3.5014	0.13
92	20609.1	2085.842	3.4755	0.43
93	20658.9	2074.941	3.4573	0.33
94	20839.1	2035.427	3.3915	0.09
95	21056.9	1987.677	3.3119	0.05
96	21197.3	1956.892	3.2606	0.29
97	21229.3	1949.889	3.2489	0.30
98	21594.6	1869.799	3.1155	0.07
99	21655.8	1856.367	3.0931	0.25
100	21715.6	1843.269	3.0713	0.64
101	21772.0	1830.901	3.0507	1.02
102	21849.2	1813.977	3.0225	0.08
103	23658.9	1417.218	2.3614	0.05
104	23691.6	1410.063	2.3495	0.07
105	23774.7	1391.839	2.3191	0.06
106	23807.6	1384.614	2.3071	0.08
107	23861.8	1372.739	2.2873	0.06
108	23899.4	1364.507	2.2736	0.08
109	23967.4	1349.586	2.2487	0.10
110	24059.8	1329.333	2.2150	0.06
111	24092.5	1322.171	2.2030	0.08
112	24199.4	1298.736	2.1640	1.43
113	24231.9	1291.603	2.1521	1.72
114	24297.2	1277.290	2.1282	0.77
115	24331.2	1269.833	2.1158	0.74
116	24371.3	1261.042	2.1012	0.72
117	24397.1	1255.385	2.0918	1.01
118	24428.7	1248.456	2.0802	0.90
119	24562.6	1219.101	2.0313	3.01
120	24626.7	1205.046	2.0079	1.13
121	24740.0	1180.214	1.9665	1.08
122	24774.4	1172.657	1.9539	0.88

			Table28.txt	
123	24818.3	1163.034	1.9379	0.34
124	24866.1	1152.568	1.9204	0.60
125	24935.7	1137.315	1.8950	0.62
126	25030.0	1116.623	1.8605	1.74
127	25069.5	1107.960	1.8461	2.53
128	25078.2	1106.053	1.8429	2.42
129	25095.8	1102.210	1.8365	3.31
130	25172.0	1085.489	1.8087	0.65
131	25231.6	1072.434	1.7869	0.82
132	25346.1	1047.339	1.7451	0.88
133	25469.2	1020.352	1.7001	0.91
134	25541.3	1004.545	1.6738	0.44
135	25577.7	996.548	1.6605	0.34
136	25610.0	989.480	1.6487	0.42
137	25640.6	982.768	1.6375	0.40
138	25673.0	975.669	1.6257	0.37
139	25707.6	968.087	1.6130	0.31
140	25760.8	956.404	1.5936	0.46
141	25838.3	939.424	1.5653	0.62
142	25922.2	921.035	1.5346	0.25
143	26005.2	902.839	1.5043	0.45
144	26091.6	883.881	1.4727	0.13
145	26262.9	846.345	1.4102	0.86
146	26302.6	837.627	1.3957	1.02
147	26356.2	825.887	1.3761	0.83
148	26497.9	794.806	1.3243	2.23
149	26575.4	777.822	1.2960	4.15
150	26638.6	763.975	1.2730	3.13
151	26725.6	744.896	1.2412	1.14
152	26768.1	735.585	1.2256	0.88
153	26797.9	729.038	1.2147	0.78
154	26834.4	721.046	1.2014	0.73
155	26864.9	714.361	1.1903	1.15
156	26917.1	702.921	1.1712	2.99
157	27005.1	683.610	1.1390	1.01
158	27056.4	672.383	1.1203	1.36
159	27114.0	659.734	1.0993	1.89
160	27148.4	652.209	1.0867	3.35
161	27218.7	636.795	1.0610	0.24
162	27370.2	603.580	1.0057	2.80
163	27473.2	580.988	0.9681	1.66
164	27504.9	574.038	0.9565	1.53
165	27560.3	561.895	0.9362	0.36
166	27579.0	557.798	0.9294	0.44
167	27785.2	512.600	0.8541	1.24
168	27863.5	495.431	0.8255	3.69
169	27956.4	475.049	0.7915	0.72
170	28020.7	460.963	0.7681	0.40
171	28053.2	453.840	0.7562	1.00
172	28088.5	446.098	0.7433	1.57
173	28120.7	439.035	0.7315	0.75
174	28191.0	423.621	0.7058	0.21
175	28213.8	418.623	0.6975	0.21
176	28292.3	401.419	0.6689	0.25
177	28332.4	392.628	0.6542	0.08
178	28707.6	310.363	0.5171	0.27
179	29201.2	202.145	0.3368	0.66
180	29348.7	169.824	0.2830	0.09
181	29371.2	164.896	0.2748	0.08
182	29508.2	134.853	0.2247	0.23
183	29515.5	133.249	0.2220	0.23

Assignment		w1	Table29.txt w2 Data Height
1C-H	110.828	6.039	32871064
2C-H	104.811	4.907	18462956
3C-H	104.435	5.323	18281274
4C-H	89.603	3.249	10669814
5C-H	85.976	4.204	13430935
6C-H	85.324	4.826	24613156
7C-H	83.319	4.948	26240892
8C-H	78.330	6.628	15279540
9C-H	78.419	4.384	16243204
10C-H	78.115	6.650	12570699
11C-H	77.405	4.786	22232300
12C-H	77.368	4.523	12061342
13C-H	76.944	4.503	11649354
14C-H	76.520	4.056	7629366
15C-H	76.506	3.946	15938518
16C-H	74.957	4.105	12325185
17C-H	75.013	4.081	13588473
18C-H	73.559	6.311	8198774
19C-H	73.017	6.229	15962149
20C-H	73.169	6.206	13342684
21C-H	72.953	4.452	17115702
22C-H	72.640	4.436	18909256
23C-H	72.679	4.405	19421648
24C-H	69.492	4.560	30325548
25C-H	67.397	4.206	20948222
26C-H	63.032	3.477	14937989
27C-H	63.051	3.457	12278297
28C-H	62.919	3.749	12207121
29C-H	62.987	3.732	11715090
30C-H	62.200	4.311	16179893
31C-H	62.213	4.287	17049402
32C-H	62.251	4.161	11319718
33C-H	61.909	4.527	17104072
34C-H	61.812	4.447	17915738
35C-H	62.056	4.132	12519210
36C-H	55.612	0.809	15916052
37C-H	55.467	0.788	15376459
38C-H	46.882	3.065	10578514
39C-H	46.931	1.687	15324549
40C-H	46.846	1.394	11274892
41C-H	46.471	3.098	6714280
42C-H	46.585	3.046	12263280
43C-H	46.664	1.410	12336944
44C-H	40.686	3.055	16128080
45C-H	38.671	1.399	11442703
46C-H	38.911	1.369	10235437
47C-H	36.670	2.153	7764437
48C-H	36.736	2.126	11053384
49C-H	36.273	2.035	8030852
50C-H	36.251	2.105	6380644
51C-H	29.592	1.503	9087281
52C-H	29.392	1.125	20473486
53C-H	29.218	1.086	101716464
54C-H	28.910	1.245	20696460
55C-H	27.910	1.290	25058176
56C-H	27.733	1.271	74552184
57C-H	27.544	1.189	22585824
58C-H	26.392	2.140	7917283
59C-H	26.158	2.099	7990846
60C-H	23.325	1.897	8840327
61C-H	22.106	0.934	8955543

Table29.txt

62C-H	20.864	1.835	101372936
63C-H	20.777	2.030	78173752
64C-H	20.638	2.003	28732000
65C-H	20.740	1.969	14511319
66C-H	20.567	1.896	14135887
67C-H	20.274	1.916	13118710
68C-H	20.357	1.746	24009208
69C-H	19.903	1.295	99321544
70C-H	19.826	1.323	50143204
71C-H	18.496	1.561	8101399
72C-H	17.189	1.004	55368712
73C-H	16.522	1.160	42240788
74C-H	16.707	1.123	30339692
75C-H	16.467	1.077	8532124
76C-H	16.634	0.968	28629992
77C-H	15.835	2.168	18934808
78C-H	15.842	2.140	16524686
79C-H	15.997	2.023	7905412
80C-H	15.370	2.098	8024050
81C-H	15.427	2.063	10964208
82C-H	15.315	1.980	12438770
83C-H	15.518	1.942	11693569
84C-H	15.430	0.848	39529776
85C-H	15.446	0.800	33285778
86C-H	11.585	0.756	13874767
87C-H	11.508	0.739	12559939
88C-H	11.480	0.706	7741734
89C-H	1.189	0.375	10674113
90C-H	1.210	0.357	11525336
91C-H	1.054	0.312	11948607
92C-H	138.517	6.066	7569417
93C-H	138.236	6.051	7116376
94C-H	138.218	5.891	6987830
95C-H	125.208	5.490	26284080

Assignment		w1	Table30.txt w2 Data Height
1H-H	6.704	6.322	9224233
2H-H	6.678	6.372	9898637
3H-H	6.419	4.456	20015776
4H-H	6.368	6.681	11333652
5H-H	6.320	6.706	11307411
6H-H	6.171	5.044	134583264
7H-H	6.043	4.952	12934876
8H-H	5.980	2.030	80531096
9H-H	5.980	2.021	105609248
10H-H	5.881	1.963	81350000
11H-H	5.881	1.952	75881512
12H-H	5.701	5.700	215204304
13H-H	5.700	5.590	18340750
14H-H	5.699	5.575	15251625
15H-H	5.582	5.701	24882492
16H-H	5.583	4.930	32200604
17H-H	5.583	4.269	54166860
18H-H	5.582	4.260	100437000
19H-H	5.582	4.251	50904084
20H-H	5.335	4.478	54908116
21H-H	5.335	4.462	48000284
22H-H	5.334	4.100	20768318
23H-H	5.048	6.174	89161792
24H-H	4.949	4.792	11766272
25H-H	4.950	4.782	9033592
26H-H	4.930	5.583	17147768
27H-H	4.929	4.269	30517720
28H-H	4.927	4.251	28975240
29H-H	4.901	4.390	27806448
30H-H	4.900	4.373	34077108
31H-H	4.831	4.790	99849200
32H-H	4.820	4.310	7275091
33H-H	4.819	4.296	7797132
34H-H	4.822	4.157	13857122
35H-H	4.821	4.138	18211468
36H-H	4.794	4.953	30630578
37H-H	4.787	4.827	86722272
38H-H	4.568	4.109	17535982
39H-H	4.567	4.093	15653847
40H-H	4.542	4.466	32239438
41H-H	4.522	4.453	145233152
42H-H	4.517	4.205	7769614
43H-H	4.523	3.952	73572400
44H-H	4.507	4.436	11835048
45H-H	4.468	5.337	79724560
46H-H	4.468	4.099	83781344
47H-H	4.461	3.954	44029948
48H-H	4.454	6.528	9453154
49H-H	4.454	6.418	79266688
50H-H	4.450	4.381	13566321
51H-H	4.449	4.321	8581714
52H-H	4.444	4.214	29697092
53H-H	4.445	4.205	23505830
54H-H	4.443	4.195	23169768
55H-H	4.382	4.902	75968808
56H-H	4.380	4.214	34194940
57H-H	4.379	4.204	34462264
58H-H	4.380	4.195	34413552
59H-H	4.302	4.823	16076232
60H-H	4.302	4.148	221634448
61H-H	4.262	5.582	80620088

Table30.txt

62H-H	4.261	4.930	59383108
63H-H	4.203	4.900	12911051
64H-H	4.204	4.433	20195584
65H-H	4.150	4.832	17371252
66H-H	4.153	4.819	18323128
67H-H	4.149	4.305	216562864
68H-H	4.099	5.337	25030224
69H-H	4.100	4.571	36121208
70H-H	4.101	4.472	68659520
71H-H	4.095	4.460	36864516
72H-H	4.028	4.468	7964866
73H-H	4.028	4.458	8388422
74H-H	3.961	4.463	41897776
75H-H	3.953	4.539	34935532
76H-H	3.952	4.523	50083884
77H-H	3.953	4.447	24637258
78H-H	3.893	1.162	59572844
79H-H	3.887	1.175	95668312
80H-H	3.766	3.516	11119055
81H-H	3.744	3.494	6884830
82H-H	3.598	3.413	146852352
83H-H	3.598	3.404	128194976
84H-H	3.517	3.767	10865892
85H-H	3.508	3.759	9535602
86H-H	3.480	3.741	8054603
87H-H	3.475	3.734	6268863
88H-H	3.409	3.599	107289744
89H-H	3.406	3.592	110786000
90H-H	3.229	2.109	12003553
91H-H	2.110	1.838	8593652
92H-H	2.107	1.822	11474128
93H-H	2.107	1.811	10279236
94H-H	2.105	1.794	8116300
95H-H	2.029	5.992	47626580
96H-H	2.027	5.984	47768532
97H-H	2.026	5.972	51874608
98H-H	2.030	2.118	7536440
99H-H	2.027	1.919	89540200
100H-H	2.024	5.964	41580316
101H-H	1.959	5.891	49355596
102H-H	1.955	5.882	46234528
103H-H	1.956	5.870	51174608
104H-H	1.954	1.848	77092128
105H-H	1.923	2.030	54674196
106H-H	1.917	2.020	54160620
107H-H	1.848	1.958	62600468
108H-H	1.834	1.756	16637570
109H-H	1.829	1.732	26336268
110H-H	1.828	1.711	15651960
111H-H	1.835	1.677	25025304
112H-H	1.830	1.553	106561752
113H-H	1.828	1.536	92597192
114H-H	1.820	3.231	15325426
115H-H	1.819	3.220	13169861
116H-H	1.818	2.114	14925469
117H-H	1.818	2.106	16020545
118H-H	1.742	1.848	24286072
119H-H	1.745	1.827	35995120
120H-H	1.669	1.827	14851102
121H-H	1.546	1.834	96039856
122H-H	1.543	1.819	72780528
123H-H	1.545	1.270	7218780
124H-H	1.546	1.255	36723868

Table30.txt

125H-H	1.544	1.241	22604678
126H-H	1.476	1.271	6846019
127H-H	1.477	1.255	10952480
128H-H	1.471	1.242	8182750
129H-H	1.474	0.727	7531610
130H-H	1.348	0.812	23199942
131H-H	1.348	0.796	27081584
132H-H	1.344	0.784	22663138
133H-H	1.260	1.540	12084268
134H-H	1.263	1.489	14416738
135H-H	1.263	1.474	17048864
136H-H	1.267	0.736	16594936
137H-H	1.266	0.719	16949332
138H-H	1.252	1.558	13171360
139H-H	1.250	1.525	19080828
140H-H	1.237	0.837	11506187
141H-H	1.167	3.901	86146744
142H-H	1.167	3.877	80709560
143H-H	0.840	1.234	8462904
144H-H	0.798	1.351	39178912
145H-H	0.798	1.342	32789168
146H-H	0.730	1.482	11378742
147H-H	0.731	1.270	11791943
148H-H	0.729	1.255	15935769
149H-H	4.460	4.531	92968608
150H-H	4.203	4.382	45014720
151H-H	4.203	4.373	32816148
152H-H	1.795	1.675	4628933

DETAILED DESCRIPTION OF THE INVENTION

There are many different periods of sleep a person goes through. These include Slow-Wave-Sleep 1 (SWS 1), Slow-Wave-Sleep 2 (SWS 2), Slow-Wave-Sleep 3 (SWS 3) Slow-Wave-Sleep 4 (SWS 4) and Rapid Eye Movement (REM). SWS 1 and SWS 2 are both periods of light sleep where it is relatively easy to wake someone up. Light sleep is usually more frequent in the second half of sleep. SWS 3 and SWS 4 are both periods of deep sleep, where it is difficult to wake the sleeper. Deep sleep is more frequent in the first half of sleep and each period will get shorter each time afterward. REM is a period of sleep in which people have their most vivid dreams. The wave patterns are similar to the patterns in which a person is awake. However, it is difficult to wake someone up who is in this state of sleep. The sleep cycle of a typical person can be described as follows:

SWS1, SWS2, SWS3, SWS4, SWS3, SWS2, REM, SWS1, SWS2, SWS3, SWS4, SWS3, SWS2, REM. (See Figure 4)

However, the above sequence may not be in a fixed order. The sleep status may shift from SWS 4 to SWS1 or wake up because the body turns from one side to the other. It may shift to the SWS2 status after the movement. The interval between two REM is about 90 min. For healthy people, SWS1 will occupy about 5% of sleep, SWS2 will occupy about 50% of sleep, SWS3 will occupy about 10% of sleep, SWS4 will occupy about 10% of sleep and REM will occupy about 25% of sleep. Since a person in SWS1 and SWS2 can easily be woken, a healthy person has enough opportunities to wake up to urinate. However if a person's sleeping status is mostly in SWS4, he has less chance of waking up when the bladder is full. It is difficult for him to break through the barriers of deep sleep. Then enuresis occurs. This invention relates to a plant extract, including Wenguanguo, for preventing enuresis.

Wenguanguo is a species of the sapindaceae family. Its scientific name is *Xanthoceras sorbifolia* Bunge. Wenguanguo is the common Chinese name; others are Wenguannguo, Wenguanmu, Wenguanhua, and Xilacedeng. This plant can grow up to 8 meters in height. It features odd pinnately compound leaf, eraceme with white flowers, capsules with thick and woody husks. Wenguanguo is grown in Liaoning, Jilin, Hebei, Shandong, Jiangsu, Henan, Shanxi, Shaanxi, Gansu, Ningxia and inner Mongolia, China. Its seeds are edible and have been used as a folk medicine to treat enuresis for centuries. Its branches and woods are also used as a folk medicine.

This invention is a further description of the extracts from Wenguanguo, their uses and methods for preparation. This invention provides the extracts that can prevent enuresis by improving patients' cerebral functions so that patients can be more aware of the signals sent from the bladder and wake up from deep sleep. When the bladder is full of urine, the smooth muscle of the bladder is extended, which produces a signal up to the cerebral cortex and cerebellum through the pelvic nerve and the sacral spinal cord. The response of the cerebral cortex and cerebellum to the signal is to make the bladder sustainably contracted but the sphincter relaxed. The urine is then discharged. When the bladder is filled with urine via the uretera during sleep, the detrusor stretches, allowing the bladder to expand. As the bladder starts to accumulate urine, it will stimulate the stretch receptors in the bladder that will generate signals continually to the brain according to the amount of urine accumulated in the bladder. When the bladder is full enough with urine, then the intravesicle has accumulated enough pressure for the brain to recognize and wake the person to urinate. If the signal is not strong enough to wake the sleeping person or blocked due to impairment of cerebral function, then enuresis occurs. This particular plant extract

can cure enuresis by improving cerebral functions.

The sensory stretch receptors are located within the bladder wall and help with assessing the degree of bladder fullness.

5 This information is transmitted up to the spinal cord and then via the spinothalamic tracts to the central nervous system. The extracts of Wenguanguo make the central nervous system more aware of the signal.

10 When the bladder becomes contracted under stress and nervousness, the capacity of the urinary bladder will be reduced and then the frequent micturition occurs. The extracts of Wenguanguo can relax the bladder for storing more urine.

15 The capacity of the urinary bladder is reduced because of aging, and this may even happen to middle-aged people. They suffer from experience of early detrusor contraction due to a sense of urgency to empty the bladder at low urine level. The extracts of Wenguanguo can help relax the detrusor and therefore the
20 bladder capacity increases and urinary frequency decreases.

Patients with detrusor overactivity, detrusor instability, detrusor hyper-reflexia or uninhibited bladder have early, forceful detrusor contractions before the bladder is full. This
25 creates urgency and frequency urinary discharge. The extract of Wenguanguo relaxes the patient's detrusor. The bladder becomes stable and can store a full amount of urine.

The smooth muscle of the urinary bladder has two functions: When
30 the bladder is relaxed, the urine is stored. When it is contracted, the urine will be discharged. The sensory stretch receptors are located within the bladder wall to assess the bladder's fullness. This information is transmitted up the spinal cord via the spinothalamic tracts to the nervous system.
35 The brain generates inhibitory signals when detrusor relaxation

is desired. But the brain generates excitatory signal when detrusor contraction is desired. The extracts of Wenguanguo can relax the bladder tissue by inhibiting Acetylcholinesterase, AchE. The inhibiting effect can be maintained for a long time.

5 The extracts of Wenguanguo are a good AChE inhibitor that can cure the diseases caused by deficiency of Acetylcholine, ACh.

Antidiuretic hormone (ADH) is stored in the posterior pituitary gland in the brain. It is the primary regulator of body water.

10 ADH acts on the kidneys to increase or decrease total body water. This has an effect on the volume of urine generated by the kidney. The release of ADH is controlled by the cells of osmoreceptors and baroreceptors. Osmoreceptors are the specialized cell hypothalamus. These cells sense the concentration of particles
15 in the blood. When the concentration of particles is higher, more ADH will be released by the pituitary. This stimulates retention of water to dilute body fluids. When the concentration is lower, less ADH will be released by the pituitary. Baroreceptors are located in the right atria and
20 great veins and carotid sinus the specialized area in the heart that sense blood volume and blood pressure. The heart will generate signals to the hypothalamus and pituitary to release more ADH when blood volume or blood pressure is low and vice versa. The extracts of Wenguanguo can regulate the release
25 of ADH which will reduce the volume of urine produced by the body.

This invention relates to the flavone extract from Wenguanguo husk and fruit-stem, and methods of their preparation. The
30 methods for preparing the extract from Wenguanguo husk and fruit-stem comprise the following steps: extracting Wenguanguo powder made from husk and fruit-stem with ethanol 3-4 times to form an ethanol extract; removing the ethanol from the ethanol extract to form an aqueous extracts; drying the aqueous extracts
35 to form the flavone extracts that is yellow powder.

This invention provides a composition comprising extracts from husk and fruit-stem which are flavonols, flavanols, dihydroflavonols, phenoloids, organic acids and others.

5

This invention relates to the crude flavone extract from Wenguanguo leaf that includes a water-soluble flavone extracts and a water-insoluble flavone extract and methods of their preparation. The methods for preparing the extract from the leaf comprise the following steps: extracting Wenguanguo powder made from the leaf with ethanol for 3 times to form an ethanol extract; concentrating the ethanol extract to form a concentrated condensed extracts; extracting the concentrated extract with hot water to form an aqueous extracts and a water-insoluble extract; drying the aqueous extracts and the water-insoluble extract to form a water-soluble flavone extracts and a water-insoluble flavone extract;. This invention provides a composition comprising the crude extracts from leaf which are flavonols, flavanols, dihydroflavonols, phenoloids, organic acids and others.

20

This invention relates to the flavone extract from Wenguanguo branch or stem and methods of their preparation. The methods for preparing the extract from branches or stem comprise the following steps: extracting Wenguanguo powder made from the branch or stem with ethanol for 4 times to form an ethanol extract; removing the ethanol from the ethanol extract to form an aqueous extracts; drying the aqueous extracts to form a flavone extracts that is yellow powder.

30

This invention provides a composition comprising extracts from branch and stem which are flavonols, flavanols, dihydroflavonols, phenoloids, organic acids and others.

35 This invention relates to the flavone extract from Wenguanguo

kernel and methods of their preparation. The methods for preparing the extract from kernel comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and drying them to form the kernel powder; extracting the kernel powder with ethanol to form an ethanol extract; removing the ethanol from the ethanol extract to form an aqueous extract; drying the aqueous extracts to form a flavone extracts that is a yellow powder.

This invention provides a composition comprising extracts from kernel which are flavonols, flavanols, dihydroflavonols, proteins, phenoloids, and others.

This invention relates to the flavone extract from Wenguanguo root, and methods of their preparation. The methods for preparing the flavone extract from Wenguanguo root comprise the following steps: extracting Wenguanguo powder made from root with ethanol 3-4 times to form an ethanol extract; removing the ethanol from the ethanol extract to form an aqueous extract; drying the aqueous extracts to form the flavone extracts that is a yellow powder.

This invention provides a composition comprising extracts from root of Wenguanguo which are flavonols, flavanols, dihydroflavonols, phenoloids, organic acids and others.

This invention relates to the flavone extract from Wenguanguo bark, and methods of their preparation. The methods for preparing the bark extract from Wenguanguo bark comprise the following steps: extracting Wenguanguo powder made from the bark with ethanol 3-4 times to form an ethanol extract; removing the ethanol from the ethanol extract to form an aqueous extract; drying the aqueous extracts to form the flavone extracts that

is a yellow powder.

5 This invention provides an extract composition from bark comprising flavonols, flavanols, dihydroflavonols, phenoloids, organic acids and others.

10 This invention is related to the combined extract from Wenguanguo husk or fruit-stem and method of their preparation. The methods for preparing the extract from the husk or fruit-stem comprise the following steps: extracting Wenguanguo powder made from the husk or fruit-stem with an organic solvent (ethanol, methanol and others) to form an organic extract; removing the organic solvent from the organic extract to form an aqueous extracts; drying and sterilizing the aqueous
15 extracts to form the combined extracts.

20 This invention provides a composition comprising the combined extracts from the husks or fruit-stem of the Wenguanguo. The combined extracts comprise saponins, saccharides, proteins and others.

25 This invention is related to the combined extract from Wenguanguo leaf and method of their preparation. The methods for preparing the extract from the leaves comprise the following steps : extracting Wenguanguo powder made from leaf with an organic solvent (ethanol, methanol and others) to form an organic extract; removing the organic solvent from the second extract to an aqueous extract; extracting the aqueous extract with ether and water to form an second aqueous extract; extracting the second aqueous extract with n-butanol to form a n-butanol extract; removing the n-butanol from the n-butanol extract to form a third aqueous extract; drying and sterilizing the third aqueous extract to form the combined extracts.

35 This invention provides a composition comprising the organic

extracts from the leaf of the Wenguanguo. The organic extracts comprise saponins, saccharides, proteins and others.

5 This invention is related to the combined extract from Wenguanguo branch or stem and method of their preparation. The methods for preparing the extract from the branch or stem comprise the following steps: extracting Wenguanguo powder made from the branch or stem with an organic solvent (ethanol, methanol and others) to form an organic extract; removing the
10 organic solvent from the second extract to an aqueous extract; drying and sterilizing the aqueous extracts to form the combined extracts.

15 This invention provides a composition comprising the organic extracts from the branch, and stem and of the Wenguanguo. The organic extracts comprise saponins, saccharides, proteins and others.

20 This invention is related to the combined extract from Wenguanguo kernel and method of their preparation. The methods for preparing the extract from the kernel comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane
25 extract and drying them to form the kernel powder; extracting the kernel powder with an organic solvent (ethanol, methanol and others) to form an organic extract; removing the organic solvent from the second extract to an aqueous extract; drying and sterilizing the aqueous extracts to form the combined
30 extracts.

35 This invention provides a composition comprising the organic extracts from the kernel of the Wenguanguo. The combined extracts comprise saponins, saccharides, proteins and others.

This invention is related to the combined extract from Wenguanguo root and method of their preparation. The methods for preparing the extract from the root comprise the following steps: extracting Wenguanguo powder made from the root with an organic solvent (ethanol, methanol and others) to form an organic extract; removing the organic solvent from the organic extract to form an aqueous extract; drying and sterilizing the aqueous extract to form the combined extract.

This invention provides a composition comprising the combined extracts from the root of the Wenguanguo. The combined extracts comprise saponins, saccharides, proteins and others.

This invention is related to the combined extract from Wenguanguo bark and method of their preparation. The methods for preparing the extract from the bark of Wenguanguo comprise the following steps: extracting Wenguanguo powder made from the bark with an organic solvent (ethanol, methanol and others) to form an organic extract; removing the organic solvent from the organic extract to form an aqueous extract; drying and sterilizing the aqueous extract to form the combined extract.

This invention provides a composition comprising the combined extracts from the bark of the Wenguanguo. The combined extracts comprise saponins, saccharides, proteins and others.

This invention provides the crude saponins from the husks or fruit-stem of Wenguanguo. The methods for preparing the crude saponins from husk or fruit-stem comprise the following steps: extracting Wenguanguo powder of the husks or fruit-stem with an organic solvent (ethanol, methanol and others) at ratio of 1:2 for 4-5 times, 20-35 hours for each time to form an organic extract; collect and reflux the organic extract 2-3 times at 80°C to form second extracts; resolve the second extracts in water to form an aqueous solution; extract the aqueous solution

by n-butanol to form a n-butanol extracts; chromatograph the n-butanol extracts to form the crude saponins.

The combined extract comprises saponins.

5

This invention provides the crude saponins from the leaf of Wenguanguo and method for their preparation. The methods for preparing the crude saponins from the leaves comprise the following steps: extracting Wenguanguo powder of the leaves with an organic solvent (ethanol, methanol and others) at ratio of 1:2, 4-5 times, 20-35 hours each time to form an organic extract; collect and reflux the organic extract 2-3 times at 80oC to form a second extract; resolve the second extracts in water to form an aqueous solution; extract the aqueous solution by n-butanol to form a n-butanol extracts; chromatograph the n-butanol extracts to form the crude saponins.

The crude extract comprises saponins.

20 This invention provides the crude saponins from the branch and stem of Wenguanguo. The methods for preparing the crude saponins from the branches or stem comprise the following steps: extracting Wenguanguo powder of the branches or stem with an organic solvent (ethanol, methanol and others) at ratio of 1:2, 4-5 times, 20-35 hours each time to form an organic extract; collect and reflux the organic extract 2-3 times at 80oC to form second extracts; resolve the second extracts in water to form an aqueous solution; extract the aqueous solution by n-butanol to form a n-butanol extracts; chromatograph the n-butanol extracts to form the crude saponins.

The crude extract comprises saponins.

35 This invention provides the crude saponins from the kernel of Wenguanguo. The methods for preparing the crude saponins from

the kernel comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and dry them to form the kernel powder; extracting the kernel powder with an organic solvent (ethanol, methanol and others) at ratio of 1:2, 4-5 times, 20-35 hours each time to form an organic extract; collect and reflux the organic extract for 2-3 times at 80°C to form second extracts; resolve the second extracts in water to form an aqueous solution; Extract the aqueous solution by n-butanol to form a n-butanol extracts; chromatograph the n-butanol extracts to form the crude saponins.

The crude extracts comprise saponins.

This invention provides the crude saponins from the root of Wenguanguo and method for their preparation. The methods for preparing the crude saponins from the root comprise the following steps: extracting Wenguanguo powder of the root with an organic solvent (ethanol, methanol and others) at ratio of 1:2, 4-5 times, 20-35 hours each time to form an organic extract; collect and reflux the organic extract 2-3 times at 80°C to form second extracts; resolve the second extracts in water to form an aqueous solution; extract the aqueous solution by n-butanol to form a n-butanol extracts; chromatograph the n-butanol extracts to form the crude saponins.

The crude extracts contain saponins.

This invention provides the crude saponins from the bark of Wenguanguo and method for their preparation. The methods for preparing the crude saponins from the bark comprise the following steps: extracting Wenguanguo powder of the bark with an organic solvent (ethanol, methanol and others) at a ratio

of 1:2, 4-5 times, 20-35 hours each time to form an organic extract; collect and reflux the organic extract 2-3 times at 80°C to form second extracts; resolve the second extracts in water to form an aqueous solution; extract the aqueous solution
5 by n-butanol to form a n-butanol extracts; chromatograph the n-butanol extracts to form the crude saponins.

The crude extracts comprise saponins.

10 This invention provides a process of producing a coumarin extract from the husk or fruit-stem of Wenguanguo and their applications. The methods for preparing the coumarin extract from husk or fruit-stem of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the husk or fruit-stem
15 with 0.5% NaOH solution to form an aqueous extract; collect and extract the aqueous extract by ether to form a ether extract; neutralize the ether extract with HCL to form a neutralized ether extract; concentrate and acidize the neutralized ether extract to form the coumarin extract.

20 This invention provides a composition comprising the coumarin extract from the husk or fruit-stem of Wenguanguo. The extract comprises coumarins, coumaric glycosides, saccharides, proteins and others.

25 This invention provides a process of producing a coumarin extract from the leaf of Wenguanguo and their applications. The methods for preparing the coumarin extract from leaves of Wenguanguo comprise the following steps: extracting Wenguanguo
30 powder of the leaves with 0.5% NaOH solution to form an aqueous extract; collect and extract the aqueous extract by ether to form a ether extract; neutralize the ether extract with HCL to form a neutralized ether extract; concentrate and acidize the neutralized ether extract to form the coumarin extract.

35

This invention provides a composition comprising the coumarin extract from the leaf of Wenguanguo. The extract comprises coumarins, coumaric glycosides, saccharides, proteins and others.

5

This invention provides a process of producing a coumarin extract from the branch and stem of Wenguanguo and their applications. The methods for preparing the coumarin extract from the branches or stem of Wenguanguo comprise the following steps: extracting Wenguanguo powder branches or stem with 0.5% NaOH solution to form an aqueous extract; collect and extract the aqueous extract by ether to form a ether extract; neutralize the ether extract with HCL to form a neutralized ether extract; concentrate and acidize the neutralized ether extract to form the extract comprising crude coumarins.

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This invention provides a composition comprising the coumarin extract from the branch and stem of Wenguanguo. The extract comprises coumarins, coumaric glycosides, saccharides, proteins and others.

20

This invention provides a process of producing a coumarin extract from the kernel of Wenguanguo and their applications. The methods for preparing the coumarin extract from the kernel of Wenguanguo comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and drying them to form the kernel powder; extracting the kernel powder with 0.5% NaOH solution to form an aqueous extract; collect and extract the aqueous extract by ether to form an ether extract; neutralizing the ether extract with HCL to form a neutralized ether extract; concentrate and acidize the neutralized ether extract to form the coumarin extract.

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This invention provides a composition comprising the coumarin extract from the kernel of Wenguanguo. The extract comprises coumarins, coumaric glycosides, saccharides, proteins and others.

5

This invention provides a process of producing a coumarin extract from the root of Wenguanguo and their applications. The methods for preparing the coumarin extract from root of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the root with 0.5% NaOH solution to form an aqueous extract; collect and extract the aqueous extract by ether to form a ether extract; neutralize the ether extract with HCL to form a neutralized ether extract; concentrate and acidize the neutralized ether extract to form the coumarin extract.

15

This invention provides a composition comprising the coumarin extract from the root of Wenguanguo. The extract comprises coumarins, coumaric glycosides, saccharides, proteins and others.

20

This invention provides a process of producing a coumarin extract from the bark of Wenguanguo and their applications. The methods for preparing the coumarin extract from bark of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the bark with 0.5% NaOH solution to form an aqueous extract; collect and extract the aqueous extract by ether to form a ether extract; neutralize the ether extract with HCL to form a neutralized ether extract; concentrate and acidize the neutralized ether extract to form the coumarin extract.

25

This invention provides a composition comprising the coumarin extract from the bark of Wenguanguo. The extract comprises coumarins, coumaric glycosides, saccharides, proteins and others.

30

This invention provides a process of producing an aqueous extract from the husk or fruit-stem of Wenguanguo and their applications. The method for preparing the water extracts from the husk or fruit-stem of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the husk or fruit-stem with water at room temperature for 24 hours to form an aqueous extract; cooking the aqueous extract at 60-70oC for 1-2 hours to form a second water extract; filtering the second water extract to form a filtered extract; concentrate the filtered extract to form the aqueous extract.

This invention provides a composition comprising the aqueous extract from the husk or fruit-stem of Wenguanguo. The aqueous extract comprises sugars, polysaccharides, glycosides, saponins, tannins and others.

This invention provides a process of producing an aqueous extract from the leaf of Wenguanguo and their applications. The method for preparing the water extracts from the leaves of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the leaves with water at room temperature for 24 hours to form an aqueous extract; cooking the aqueous extract at 60-70oC for 1-2 hours to form a second water extract; filtering the second water extract to form a filtered extract; concentrate the filtered extract to form the aqueous extract.

This invention provides a composition comprising the aqueous extract from leaf of Wenguanguo. The aqueous extract comprises sugars, polysaccharides, glycosides, saponins, tannins and others.

This invention provides a process of producing an aqueous extract from the branch or stem of Wenguanguo and their applications. The method for preparing the water extracts from branches or stem of Wenguanguo comprise the following steps:

extracting the Wenguanguo powder of the branches or stem with water at room temperature for 24 hours to form an aqueous extract; cooking the aqueous extract at 60-70°C for 1-2 hours to form a second water extract; filtering the second water extract to
5 from a filtered extract; concentrating the filtered extract to form the aqueous extract.

This invention provides a composition comprising the aqueous extract from the branch or stem of Wenguanguo. The aqueous
10 extract comprises sugars, polysaccharides, glycosides, saponins, tannins and others.

This invention provides a process of producing an aqueous extract from the kernel of Wenguanguo and their applications.
15 The method for preparing the water extracts from the kernel of Wenguanguo comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and
20 dry them to form the kernel powder; extracting the kernel powder with water at room temperature for 24 hours to form an aqueous extract; cooking the aqueous extract at 60-70°C for 1-2 hours to form a second water extract; filtering the second water extract to form a filtered extract; concentrate the filtered
25 extract to form the aqueous extract.

This invention provides a composition comprising the aqueous extract from kernel of Wenguanguo. The aqueous extract
30 comprises sugars, polysaccharides, glycosides, saponins, tannins and others.

This invention provides a process of producing an aqueous extract from the root of Wenguanguo and their applications. The
35 method for preparing the water extracts from the root of Wenguanguo comprises the following steps: extracting

Wenguanguo powder of the root with water at room temperature for 24 hours to form an aqueous extract; cooking the aqueous extract at 60-70°C for 1-2 hours to form a second water extract; filtering the second water extract to form a filtered extract; 5 concentrating the filtered extract to form the aqueous extract.

This invention provides a composition comprising the aqueous extract from the root of Wenguanguo. The aqueous extract comprises sugars, polysaccharides, glycosides, saponins, 10 tannins and others.

This invention provides a process of producing an aqueous extract from the bark of Wenguanguo and their applications. The method for preparing the water extracts from the bark of 15 Wenguanguo comprise the following steps: extracting Wenguanguo powder of the bark with water at room temperature for 24 hours to form an aqueous extract; cooking the aqueous extract at 60-70°C for 1-2 hours to form a second water extract; filtering the second water extract to form a filtered extract; concentrate 20 the filtered extract to form the aqueous extract.

This invention provides a composition comprising the aqueous extract from the bark of Wenguanguo. The aqueous extract comprises sugars, polysaccharides, glycosides, saponins, 25 tannins and others.

This invention provides a process of producing an alkaloid extract from the husk of Wenguanguo and their applications. The methods for preparing the alkaloid extract from the husk and 30 fruit-stem of Wenguanguo comprising the following steps: extracting Wenguanguo powder of the husk or fruit-stem with water at a ratio of 1:6, 3-4 times, 10-15 hours each time to form an aqueous extract; collect and alkalify the aqueous extract with NaOH to form a alkalified aqueous extract with pH 35 10-12; extract the alkalified aqueous extract by toluol to form

a toluol extract; the toluol extract flows through 2% of dicarboxyl solution with pH 5-7 to form a dicarboxyl solution; concentrate the dicarboxyl solution with decompression a to form crude alkaloids.

5

This invention provides a composition comprising the alkaloid extract from the husk or fruit-stem of Wenguanguo. The extract comprises alkaloids, coumarins, saccharides, proteins and others.

10

This invention provides a process of producing an alkaloid extract from the leaf of Wenguanguo and their applications. The methods for preparing the alkaloid extract from the leaves of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the leaves with water at a ratio of 1:6, 3-4 times, 10-15 hours each time to form an aqueous extract; collecting and alkalifying the aqueous extract with NaOH to form a alkalified aqueous extract with pH 10-12; extracting the alkalified aqueous extract by toluol to form a toluol extract; flow the toluol extract through 2% of dicarboxyl solution with pH 5-7 to form a dicarboxyl solution; concentrate the dicarboxyl solution with decompression to form the alkaloid extract.

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This invention provides a composition comprising the alkaloid extract from the leaf of Wenguanguo. The extract comprises alkaloids, coumarins, saccharides, proteins and others.

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This invention provides a process of producing an alkaloid extract from the branch and stem of Wenguanguo and their applications. The methods for preparing the extracts containing alkaloids from branches or stem of Wenguanguo comprising the following steps: extracting Wenguanguo powder of the branches or stem with water at ratio of 1:6, 3-4 times, 10-15 hours each time to form an aqueous extract; collect and alkalify the aqueous extract with NaOH to form a alkalified

aqueous extract with pH 10-12; extracting the alkalified aqueous extract by toluol to form a toluol extract; flow the toluol extract through 2% of dicarboxyl solution with pH 5-7 to form a dicarboxyl solution; concentrate the dicarboxyl solution with decompression to form the alkaloid extract.

This invention provides a composition comprising the extract containing crude alkaloids from the branch or stem of Wenguanguo. The extract comprises alkaloids, coumarins, saccharides, proteins and others.

This invention provides a process of producing an alkaloid extract from the kernel of Wenguanguo and their applications. The methods for preparing the alkaloid extract from kernel of Wenguanguo comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grounding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and dry them to form the kernel powder; extracting the kernel powder with water at ratio of 1:6 for 3-4 times, 10-15 hours for each time to form an aqueous extract; collect and alkalify the aqueous extract with NaOH to form a alkalified aqueous extract with pH 10-12; extract the alkalified aqueous extract by toluol to form a toluol extract; the toluol extract flows through 2% of dicarboxyl solution with pH 5-7 to form a dicarboxyl solution; concentrate the dicarboxyl solution with decompression to form the alkaloid extract.

This invention provides a composition comprising the alkaloid extract from the kernel of Wenguanguo. The extract comprises alkaloids, coumarins, saccharides, proteins and others.

This invention provides a process of producing an alkaloid extract from the root of Wenguanguo and their applications. The methods for preparing the alkaloid extract from the root of

Wenguanguo comprise the following steps: extracting Wenguanguo powder of the Wenguanguo root with water at a ratio of 1:6, 3-4 times, 10-15 hours each time to form an aqueous extract; collecting and alkalifying the aqueous extract with NaOH to form
5 a alkalified aqueous extract with pH 10-12; extracting the alkalified aqueous extract by toluol to form a toluol extract; flow the toluol extract through 2% of dicarboxyl solution with pH 5-7 to form a dicarboxyl solution; concentrate the dicarboxyl solution with decompression a to form crude alkaloids.

10

This invention provides a composition comprising the alkaloid extract from the root of Wenguanguo. The extract comprises alkaloids, coumarins, saccharides, proteins and others.

15

This invention provides a process of producing an alkaloid extract from the bark of Wenguanguo and their applications. The methods for preparing the alkaloid extract from the bark of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the bark with water at ratio of 1:6, 3-4 times, 10-15
20 hours each time to form an aqueous extract; collect and alkalify the aqueous extract with NaOH to form a alkalified aqueous extract with pH 10-12; extract the alkalified aqueous extract by toluol to form a toluol extract; flow the toluol extract through 2% of dicarboxyl solution with pH 5-7 to form a
25 dicarboxyl solution; concentrate the dicarboxyl solution with decompression a to form crude alkaloids.

This invention provides a composition comprising the alkaloid extract from the bark of Wenguanguo. The extract comprises
30 alkaloids, coumarins, saccharides, proteins and others.

This invention provides a process of producing extract containing organic acids from husk and fruit-stem and their applications. The methods for preparing the extracts containing
35 organic acids from the husk or fruit-stem of Wenguanguo comprise

the following steps: extract Wenguanguo powder of the husk and or fruit-stem with 10% HCL to form an acid solution; extract the acid solution by an organic solvent (ether or benzol) to form organic extract; extract the organic extract by 5-10% NaOHCO₃ solution to form a NaOHCO₃ extract; acidize and filter the NaOHCO₃ extract to form a deposit matter; extract the deposit matter by an organic solvent to form the second organic extract; remove the organic solvent from the second extract to form crude organic acid.

This invention provides a composition comprising crude organic acids from the husk of Wenguanguo. The extract comprising aromatic organic acids, fatty organic acids, terpenoid organic acids, saccharides, proteins and others

This invention provides a process of producing extract contains organic acids from leaf and their applications. The methods for preparing the extracts containing organic acids from the leaves of Wenguanguo comprise the following steps: extract Wenguanguo powder of the leaves with 10% HCL to form an acid solution; extract the acid solution by an organic solvent (ether or benzol) to form organic extract; extract the organic extract by 5-10% NaOHCO₃ solution to form a NaOHCO₃ extract; acidize and filter the NaOHCO₃ extract to form a deposit matter; extract the deposit matter by an organic solvent to form the second organic extract; remove the organic solvent from the second extract to form crude organic acid.

This invention provides a composition comprising the extract comprising crude organic acids extract from the leaf of Wenguanguo. The extract comprises aromatic organic acids, fatty organic acids, terpenoid organic acids, saccharides, proteins and others.

This invention provides a process of producing extract contains

organic acids from branch and stem and their applications. The methods for preparing the extracts comprising organic acids from the branches or stem of Wenguanguo comprise the following steps: extract Wenguanguo powder of the branches or stem with
5 10% HCL to form an acid solution; extract the acid solution by an organic solvent (ether or benzol) to form organic extract; extract the organic extract by 5-10% NaOHCO₃ solution to form a NaOHCO₃ extract; acidize and filter the NaOHCO₃ extract to form a deposit matter; extract the deposit matter by an organic
10 solvent to form the second organic extract; remove the organic solvent from the second extract to form crude organic acid.

This invention provides a composition comprising the crude organic acids extract from the branch and stem of Wenguanguo.
15 The extract comprises aromatic organic acids, fatty organic acids, terpenoid organic acids, saccharides, proteins and others.

This invention provides a process of producing extract comprise
20 organic acids from kernel and their applications. The methods for preparing the extracts comprising organic acids from the kernel of Wenguanguo comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grounding and extracting the kernel cakes with n-hexane to form n-hexane
25 extract; removing the n-hexane from the n-hexane extract and dry them to form the kernel powder; extracting the kernel powder with 10% HCL to form an acid solution; extract the acid solution by an organic solvent (ether or benzol) to form organic extract; extract the organic extract by 5-10% NaOHCO₃ solution to form
30 a NaOHCO₃ extract; acidize and filter the NaOHCO₃ extract to form a deposit matter; extract the deposit matter by an organic solvent to form the second organic extract; remove the organic solvent from the second extract to form crude organic acid.

35 This invention provides a composition comprising crude organic

acids extract from the kernel of Wenguanguo. The extract comprises aromatic organic acids, fatty organic acids, terpenoid organic acids, saccharides, proteins and others.

5 This invention provides a process of producing extract contains organic acids from the root of Wenguanguo and their applications. The methods for preparing the extracts containing organic acids from the root of Wenguanguo comprise the following steps: extract Wenguanguo powder of the root with 10% HCL to form an
10 acid solution; extract the acid solution by an organic solvent (ether or benzol) to form organic extract; extract the organic extract by 5-10% NaOHCO₃ solution to form a NaOHCO₃ extract; acidize and filter the NaOHCO₃ extract to form a deposit matter; extract the deposit matter by an organic solvent to form the
15 second organic extract; remove the organic solvent from the second extract to form crude organic acid.

This invention provides a composition comprising the extract comprising crude organic acids from the root of Wenguanguo. The
20 extract comprises aromatic organic acids, fatty organic acids, terpenoid organic acids, saccharides, proteins and others.

This invention provides a process of producing extract comprising organic acids from bark of Wenguanguo and their
25 applications. The methods for preparing the extracts containing organic acids from the bark of Wenguanguo comprise the following steps: extract Wenguanguo powder of the bark with 10% HCL to form an acid solution; extract the acid solution by an organic solvent (ether or benzol) to form organic extract;
30 extract the organic extract by 5-10% NaOHCO₃ solution to form a NaOHCO₃ extract; acidize and filter the NaOHCO₃ extract to form a deposit matter; extract the deposit matter by an organic solvent to form the second organic extract; remove the organic solvent from the second extract to form crude organic acid.

35

This invention provides a composition comprising the extract comprising crude organic acids from the bark of Wenguanguo. The extract comprises aromatic organic acids, fatty organic acids, terpenoid organic acids, saccharides, proteins and others.

5

This invention provides two methods of producing a tannin extract from Wenguanguo husk and fruit-stem and its usage. The method-1 for preparing the tannin extract from the husk or fruit-stem of Wenguanguo comprises the following steps:

10

extracting Wenguanguo powder of husk and or fruit-stem with 95% ethanol to form an ethanol extract; concentrate the ethanol extract with decompression a to form the tannin extract. The

method-2 for preparing the tannin extract from the husk and or fruit-stem of Wenguanguo comprise the following steps:

15

extracting Wenguanguo powder of the husk and or fruit-stem with a solvent of acetone-water at ratio of 1:1 for 2-7 days to form an acetone-water extract; removing acetone from the acetone-water extract at 50oC to form a concentrated extract;

filtering the concentrated extract to form a filtered extract;

20

extract the filtered extract with ether to form an aqueous extract; extracting the aqueous extract with ethyl acetate and n-butanol to form ethyl acetate and n-butanol extract comprising tannins.

25

This invention provides a composition comprising the tannin extracts from the husk or fruit-stem of Wenguanguo. The extracts are comprised of tannins, organic acids, saccharides, proteins and others.

30

This invention provides two methods of producing a tannin extract from Wenguanguo leaf and its usage. The method-1 for preparing the tannin extract from the leaves of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the leaves with 95% ethanol to form an ethanol extract;

35

concentrate the ethanol extract with decompression a to form

the tannin extract.

5 The method-2 for preparing the tannin extract from the leaves of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the leaves with a solvent of acetone-water at ratio of 1:1 for 2-7 days to form an acetone-water extract; removing acetone from the acetone-water extract at 50oC to form a concentrated extract; filtering the concentrated extract to form a filtered extract; extract the filtered extract with ether to form an aqueous extract; extracting the aqueous extract with ethyl acetate and n-butanol to form ethyl acetate and n-butanol extract containing tannins.

15 This invention provides a composition comprising the tannin extract from the leaf of Wenguanguo. The extract comprises tannins, organic acids, saccharides, proteins and others.

20 This invention provides two methods of producing a tannin extract from Wenguanguo branch and stem and its usage. The method-1 for preparing the extracts comprising tannins from branch or stem of Wenguanguo comprise the following steps: extracting Wenguanguo powder of branches or stem with 95% ethanol to form an ethanol extract; concentrate the ethanol extract with decompression a to form the tannin extract.

25 The method-2 for preparing the tannin extract from the branches or stem of Wenguanguo comprise the following steps: extracting Wenguanguo powder of the branches or stem and with a solvent of acetone-water at ratio of 1:1 for 2-7 days to form an acetone-water extract; removing acetone from the acetone-water extract at 50oC to form a concentrated extract; filtering the concentrated extract to form a filtered extract; extract the filtered extract with ether to form an aqueous extract; extracting the aqueous extract with ethyl acetate and n-butanol to form ethyl acetate and n-butanol extract comprising tannins.

This invention provides a composition comprising the tannin extract from the branch or stem of Wenguanguo. The extract comprises tannins, organic acids, saccharides, proteins and others.

5

This invention provides two methods of producing a tannin extract from Wenguanguo kernel and its usage. The method-1 for preparing the tannin extract from the kernel of Wenguanguo comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and dry them to form the kernel powder; extracting the kernel powder with 95% ethanol to form an ethanol extract; concentrating the ethanol extract with decompression to form the extract comprising tannins.

The method-2 for preparing the extracts containing tannins from the kernel of Wenguanguo comprise the following steps: removing oil by pressing the kernel to form kernel cakes; grinding and extracting the kernel cakes with n-hexane to form n-hexane extract; removing the n-hexane from the n-hexane extract and dry them to form the kernel powder; extracting the kernel powder with a solvent of acetone-water at ratio of 1:1 for 2-7 days to form an acetone-water extract; removing acetone from the acetone-water extract at 50°C to form a concentrated extract; filtering the concentrated extract to form a filtered extract; extracting the filtered extract with ether to form an aqueous extract; extracting the aqueous extract with ethyl acetate and n-butanol to form ethyl acetate and n-butanol extract containing tannins.

This invention provides a composition comprising the tannin extract from kernel of Wenguanguo. The extract comprises tannins, organic acids, saccharides, proteins and others.

35

This invention provides two methods of producing a tannin extract from Wenguanguo root and its usage. The method-1 for preparing the tannin extract from the root of Wenguanguo comprises the following steps: extracting Wenguanguo powder of
5 root with 95% ethanol to form an ethanol extract; concentrating the ethanol extract with decompression to form the tannin extract. The method-2 for preparing the tannin extract from the root of Wenguanguo comprises the following steps: extracting Wenguanguo powder of the root with a solvent of acetone-water
10 at a ratio of 1:1 for 2-7 days to form an acetone-water extract; removing acetone from the acetone-water extract at 50oC to form a concentrated extract; filtering the concentrated extract to form a filtered extract; extracting the filtered extract with ether to form an aqueous extract; extracting the aqueous extract
15 with ethyl acetate and n-butanol to form ethyl acetate and n-butanol extract comprising tannins.

This invention provides a composition comprising the tannin extracts from the root of Wenguanguo. The extracts comprise
20 tannins, organic acids, saccharides, proteins and others.

This invention provides two methods of producing a tannin extract from Wenguanguo bark and its usage. The method-1 for preparing the tannin extract from the bark of Wenguanguo
25 comprises the following steps: extracting Wenguanguo powder of bark with 95% ethanol to form an ethanol extract; concentrating the ethanol extract with decompression to form the tannin extract. The method-2 for preparing the tannin extract from the bark of Wenguanguo comprising the following steps: extracting
30 Wenguanguo powder of the bark with a solvent of acetone-water at ratio of 1:1 for 2-7 days to form an acetone-water extract; removing acetone from the acetone-water extract at 50oC to form a concentrated extract; filtering the concentrated extract to form a filtered extract;
35 extracting the filtered extract with ether to form an aqueous

extract; extracting the aqueous extract with ethyl acetate and n-butanol to form ethyl acetate and n-butanol extract comprising tannins.

- 5 This invention provides a composition comprising the tannin extracts from the bark of Wenguanguo. The extracts comprise tannins, organic acids, saccharides, proteins and others.

10 This invention provides a method for preventing cerebral aging, improving memory, improving cerebral functions and curing enuresis, frequent micturition, urinary incontinence, dementia, weak intelligence and Alzheimer's disease, autism, brain trauma, Parkinson's disease and other diseases caused by cerebral dysfunctions, and treating arthritis, rheumatism, poor
15 circulation, arteriosclerosis, Raynaud's syndrome, angina pectoris, cardiac disorder, coronary heart disease, headache, dizziness, kidney disorder and treating impotence and premature ejaculation.

- 20 This invention provides the medicines or health foods which further comprise Vitamin B, Vitamin D, Vitamin K, grape seed extract and other antioxidants, Cordyceps, or its extract, ginkgo, or its extract, Panax ginseng and P. quinquefolium or their extracts, Huangpi (*Clausena lansium*), or its extracts,
25 Echinacea or its extract, St John's Wort (*Hypericum perforatum*), or its extract, Gegen (*Pueraria lobata*), or its extract, Tianma (*Gastrodia elata*), or its extract, Armillariella mellea, or its extract, Danshen (*Salvia miltiorrhiza*), or its extract, Sanqi (*Panax notoginseng*), or its extract, Monascus or Honqu (Red
30 yeast rice), Huanqi (*Hedysarum polybotrys*), or its extract, D ihuang (*Rehmannia glutinosa*), or its extract, Danggui (*Angelica sinensis*), Yuanzhi (*Polygala tenuifolia*), or its extract, Lingzhi (*Ganoderma spp.*), or its extracts, Fuling (*Poria cocos*), or its extract, enokitake (*Flammulina velutipes*), or its
35 extract, Gan Cao (*Glycyrrhiza uralensis* Fisch) or its extract,

Huperzine A, Lacithin, Metrifonate, Nocetile, folic acid, amino acids, creatine, fiber supplement, or any combination thereof.

5 This invention provides the extract of wenguanguo for inhibiting the uptake of 5-hydroxytryptamine (5HT) in a subject.

10 5-HT controls and modulates a sleep factor that sustains and increases deep sleep. Inhibiting the uptake of 5HT will decrease deep sleep. People who spend too much time in SWS 3 and SWS 4 are unable to awaken from their sleep when their bladder is full because their sleep is too deep. This is the reason that enuresis often occurs during SWS 3 and SWS 4.

15 This invention provides the extract of wenguanguo for increasing the activity of Dopamine in a subject thereby making the central nerve system of said subject alert.

20 This invention provides the extract of wenguanguo for increasing the secretion of Antidiuretic hormone (ADH) in a subject, which reduces urine in said subject.

25 This invention provides the extract of wenguanguo for modulating the release, breakdown and uptake of Acetylcholine (Ach) and its receptors in a subject. The said extracts of this invention inhibits the deep sleep created by 5HT and increase REM sleep.

30 This invention provides the extract of wenguanguo for preventing sleep paralysis in a subject.

This invention provides the extract of wenguanguo for providing alertness to a sleeping subject.

35 This invention provides the extract for helping the growth of

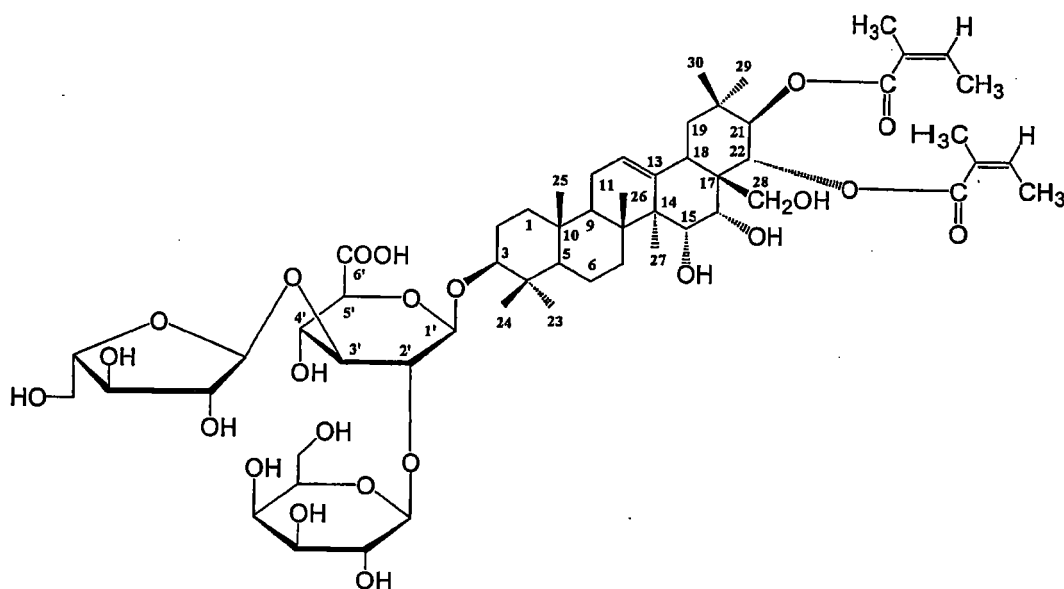
the bladder and sphincter.

An immature bladder and sphincter cannot control the process and action of urination. By accelerating the growth of the bladder and the sphincter, this problem will be overcome, and enuresis will not occur.

This invention provides the extract of wenguanguo against cancer growth. The cancer includes, but is not limited to bladder cancer, cervix cancer, prostate cancer, lung cancer, breast cancer, leukocytes cancer, colon cancer, liver cancer, bone cancer, brain cancer, and ovary cancer.

This invention provides a compound comprising a sugar, a terpenene or Sapogenin, and a side chain at Carbon 21 and 22 or Angeloyl groups. In an embodiment, the compound comprises two or more sugars.

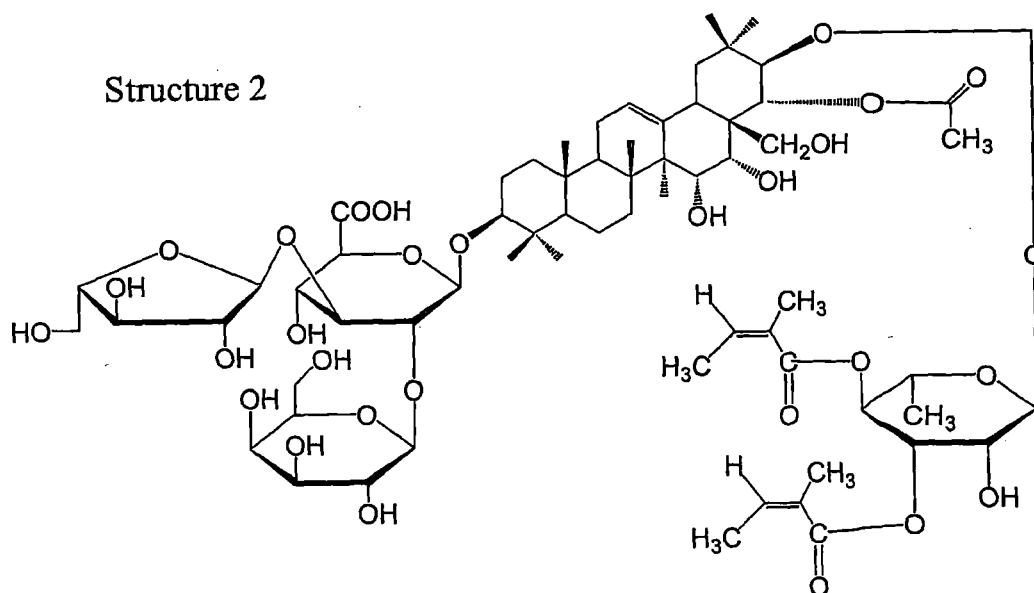
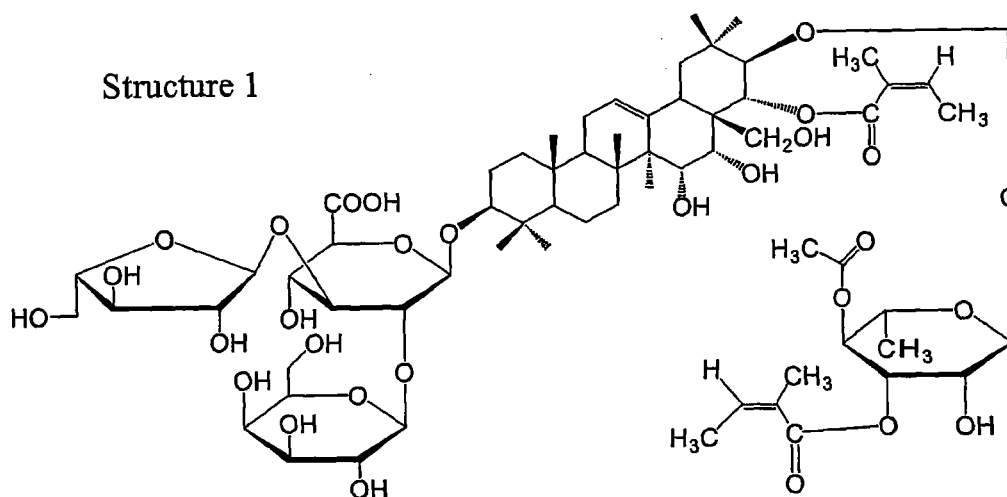
This invention provides a compound comprising the following structure:



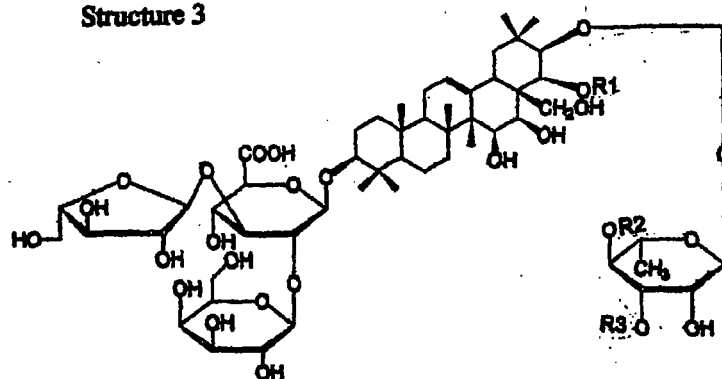
3-O- [β-D-galactopyranosyl (1→2)] -α-L-arabinofuranosyl (1→3) -

β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene, also known as Xantho-b1

In other embodiments, the structures of the compounds are as follows:



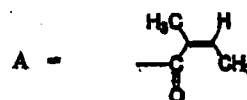
Structure 3



Where R1 = A or B or C

R2 = A or B or C

R3 = A or B or C

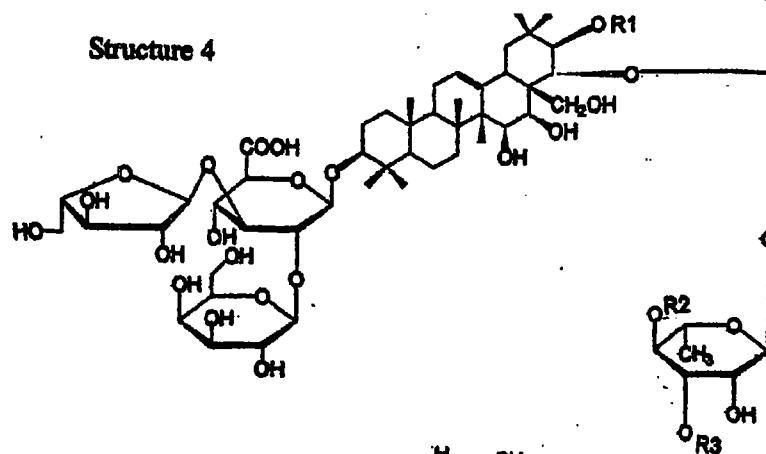


	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

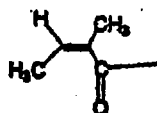
10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

Structure 4



A =



Where R1 = A or B or C

R2 = A or B or C

R3 = A or B or C

B =



C =

H

	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

This invention provides a salt of the above-described compounds.

This invention provides a composition comprising the
5 above-described compounds and a suitable carrier.

This invention provides a pharmaceutical composition comprising an effective amount of the above-described compounds

and a pharmaceutically acceptable carrier.

This invention provides an anti-ovarian cancer and composition comprising the above-described composition.

5

The following methods and materials were used in the examples/experiments described below:

10 Cells. Human cancer cell lines were obtained from American Type Culture Collection: HTB-9 (bladder), HeLa-S3 (cervix), DU145 (prostate), H460 (lung), MCF-7 (breast), K562 (leukocytes), HCT116 (colon), HepG2 (liver), U2OS (bone), T98G (brain) and OVCA-3 (ovary). Cells were grown in culture medium (HeLa-S3, DU145, MCF-7, Hep-G2 and T98G in MEM (Earle's salts); HTB-9, 15 H460, K562, OVCA-3 in RPMI-1640; HCT-116, U2OS in McCoy-5A) supplemented with 10% fetal calf serum, glutamine and antibiotics in a 5% CO₂ humidified incubator at 37°C.

20 MTT Assay. The procedure for MTT assay followed the method described in (Carmichael et al., 1987) with only minor modifications. Cells were seeded into a 96-wells plate at concentrations of 10,000/well (HTB-9, HeLa, H460, HCT116, T98G, OVCA-3), 15,000/well (DU145, MCF-7, HepG2, U2OS), or 40,000/well (K562), for 24 hours before drug-treatment. Cells 25 were then exposed to drugs for 48 hours (72 hours for HepG2, U2OS, and 96 hours for MCF-7). After the drug-treatment, MTT (0.5 mg/ml) was added to cultures for an hour. The formation of formazan (product of the reduction of tetrazolium by viable cells) was dissolved with DMSO and the O.D. at 490nm was measured 30 by an ELISA reader. The MTT level of cells before drug-treatment was also measured (T₀). The % cell-growth (%G) is calculated as:

$$\begin{array}{lcl} \%G & = & (TD - T_0) / (TC - T_0) \times 100, \\ 35 & (1) & \end{array}$$

where TC or TD represent O.D. readings of control or drug-treated cells.

When $T0 > TD$, then the cytotoxicity (LC) expressed as % of the control is calculated as:

$$\%LC = \frac{(TD - T0)}{T0} \times 100.$$

(2)

Fractionation and Analysis. 0.5 g of the extract powder was partitioned between H₂O (200ml) and N-butanol (200ml). The butanol fraction was freeze dried and two thirds of the residue was placed onto a pre-packed 5 g RPC18IST column pre-wet with MeCN, then MeCN: H₂O then H₂O (5ml each). The column was developed in stages from H₂O through to MeCN. The fractions were examined by silica gel TLC. The fourth, fifth and sixth fraction, eluted with H₂O: MeCN (80:20 and 50: 50), showed TLC spots around R_f 0.11, contain saponins.

The H NMR spectra (CD₃OD solution, 300MHz) on these fractions showed signals in the region of 3-4ppm which are typical of glycosides, and signals in region 0.82-2 ppm typical of the aglycone portion of saponins.

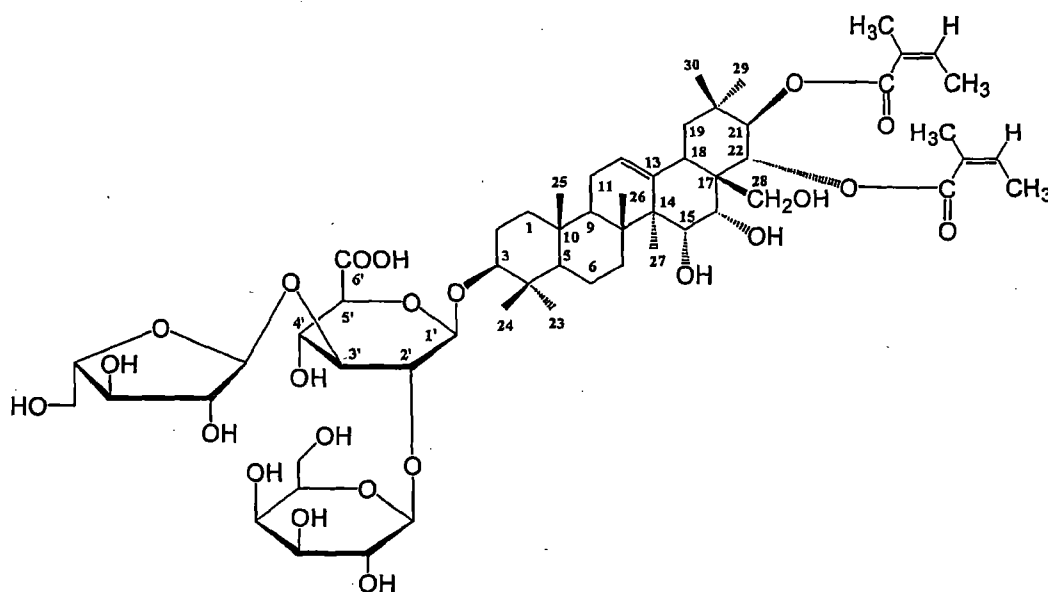
The H NMR spectra of fraction 4 also contained signals in the region 5.8-7.8 ppm which could be due to coumarins and flavonoids. The spectra of fractions 5 and 6 were mostly similar to one another. Fractions 4, 5, 6 were contain saponins. The appearance and NMR spectra are indicative of saponins.

TLC Procedure. The fractions from the RP C18 IST columns were prepared at a standard concentration (10 mg/ml) and each fraction was spotted onto a silica gel TLC plate (Merck Keiselgel 60 F254). This was developed using the bottom layer of the following mixture as a mobile phase (CHCl₃: MeOH: H₂O-35:65:40). The separated spots were visualised using UV

light and acidified vanillin dip(2g vanillin, in 100 ml EtOH plus H₂SO₄, 1ml)

This invention provides the pathways interacted by compounds isolated from *Xanthoceras Sorbifolia*. In an embodiment, a compound has the formula C₅₇H₈₈O₂₃ and the chemical name 3-O- [β -D-galactopyranosyl (1 $\rightarrow$ 2)] - α -L-arabinofuranosyl (1 $\rightarrow$ 3) - β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene, and derivative compounds which are effective against cancer. They regulate the receptors or components of cells. The compounds can be isolated from the plant called *Xanthoceras Sorbifolia* or can be synthesized.

The compound is:

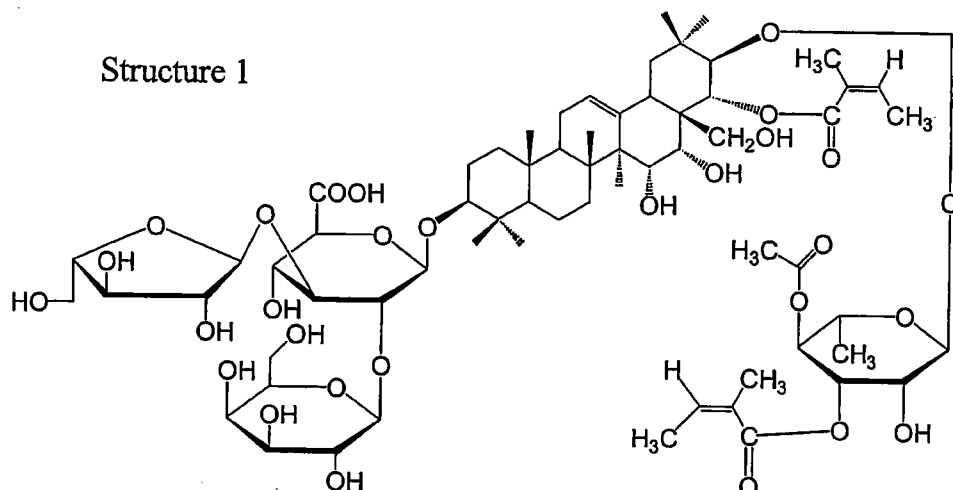


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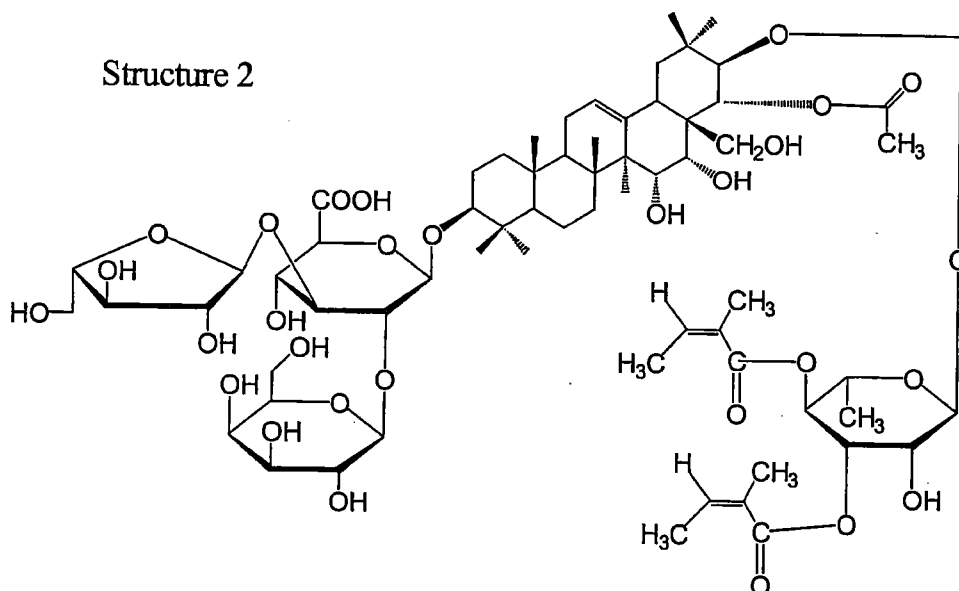
3-O- [β -D-galactopyranosyl (1 $\rightarrow$ 2)] - α -L-arabinofuranosyl (1 $\rightarrow$ 3) - β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene

20

Structure 1



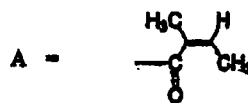
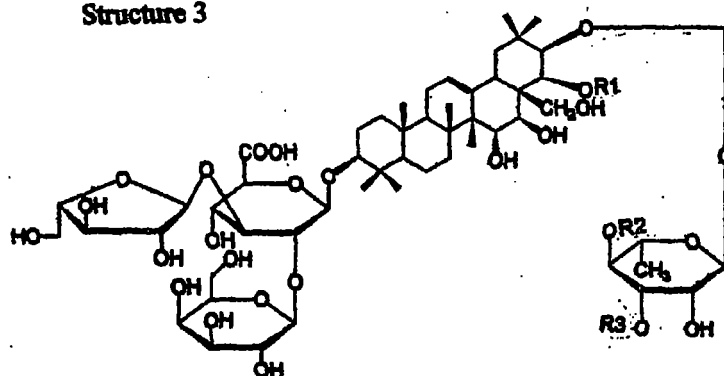
Structure 2



5

10

Structure 3



Where R1 = A or B or C

R2 = A or B or C

$$B = \begin{array}{c} \text{H}_3\text{C} - \text{C} \\ | \\ \text{C} \end{array}$$

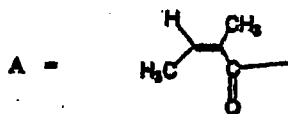
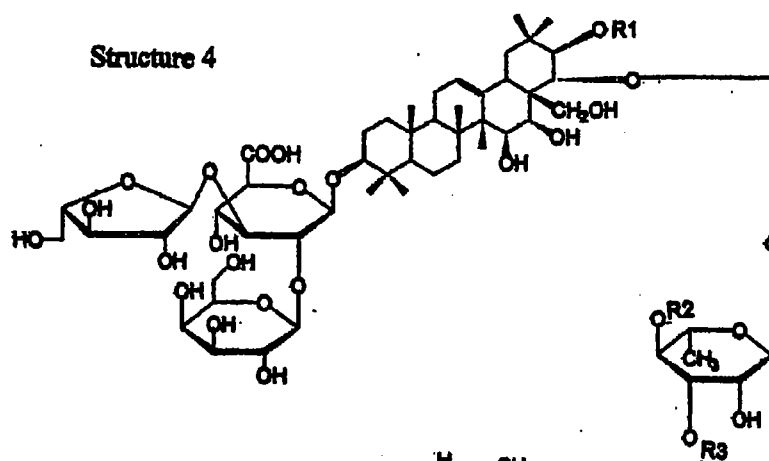
R3 = A or B or C

C - H

	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C



Where R1 = A or B or C

R2 = A or B or C

R3 = A or B or C



	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

There are many components and pathways monitoring cell proliferation.

5 The Xanthoceras Sorbifolia compound or its derivatives work in the Wnt (Wingless-type MMTV integration site family member) signaling pathway. The Wnt signaling pathway is evolutionarily conserved and controls many events during the embryogenesis.

This pathway regulates cell morphology, proliferation, motility and as well as cell apoptosis. It also plays an important role during tumorigenesis. The Wnt pathway has also been observed as inappropriately activated in several different
5 types of cancers in humans.

In the nucleus, the target genes for Wnt signaling are normally kept silent by an inhibitory complex of gene regulatory proteins, e.g. the Groucho corepressor protein bound to the gene
10 regulatory protein LEF-1/TCF. In the absence of a Wnt signal, some β -catenin is bound to the cytosolic tail of cadherin proteins, and any cytosolic β -catenin that becomes bound by the APC-axin-GSK-3 β will trigger its ubiquitylation and degradation in proteasomes. The result is the decrease of
15 intracellular amount of β -catenin. However, when the Wnt binding to Frizzled (a seven transmembrane receptor) and LRP (Low density lipoprotein Receptor) activates Dishevelled (a cytoplasmic signaling protein) by a mechanism, this leads to the inactivation of GSK-3 β in the degradation complex by a mechanism
20 which requires casein kinase I, as well as casein kinase II. The activity of the multiprotein complex of β -catenin-axin-adenomatous-polyposis coli (APC)-glycogen synthase kinase (GSK)-3 β , which targets β -catenin by phosphorylation for degradation by the proteasome, is then
25 inhibited by Dsh/Dvl (Dishevelled, dsh homolog 1). This then inhibits priming of β -catenin, and indirectly prevents the GSK-3 β phosphorylation of β -catenin. When stimulated by Wnt, Dvl recruits the GSK-3 binding protein, GBP, to the multiprotein complex of β -catenin-axin-adenomatous-polyposis coli
30 (APC)-glycogen synthase kinase (GSK)-3 β . GBP then titrates GSK-3 β from axin, and in this way, phosphorylation of β -catenin is inhibited. Then, axin is sequestered by LRP at the cell membrane. The result of all of this is an accumulation of cytosolic β -catenin. In the nucleus, β -catenin binds to
35 LEF-1/TCF, displaces Groucho, and acts a co-activator to

stimulate the transcription of Wnt target genes.

Xanthoceras Sorbifolia compositions regulate the components related to Wnt pathways or its receptors, thereby stopping the proliferation of cancer cells.

The compound or its derivatives work in the Mitogens, Ras and a MAP (Microtubule associated protein) kinase pathway. Mitogens stimulate cell division. The binding of mitogens to cell-surface receptors leads to the activation of Ras and a MAP kinase cascade. One effect of this pathway is the increased production of the gene regulatory protein Myc. Myc increases the transcription of several genes, including the gene encoding cyclin D and a subunit of the SCF ubiquitin ligase. The resulting increase in G₁-Cdk and G₁/S-Cdk activities promotes Rb phosphorylation and activation of the gene regulatory protein E2F, resulting in S-phase entry, in which G₁-Cdk activity initiates Rb phosphorylation, in turn inactivating Rb and freeing E2F to activate the transcription of S-phase genes including the genes for a G₁/S-cyclin(cyclin E) and S-cyclin(cyclin A). The resulting appearance of G₁/S-Cdk and S-Cdk further enhances Rb phosphorylation, forming a positive feedback loop, and the E2F acts back to stimulate the transcription of its own gene, forming another positive feedback loop. Myc may also promote E2F activity directly by stimulating the transcription of the E2F gene. The result is the increased transcription of genes entry into S phase. However if this pathway is overactive, it will cause cancer cell growth.

Compounds or compositions derived from the plant Xanthoceras Sorbifolia regulate the Ras-MAP kinase cascade so that the pathway is not overactive.

The compound or its derivatives work in Ras-dependent or Myc pathway. Sometimes the mutation of amino acid in Ras causes the

protein to become permanently overactive, stimulating the Ras-dependent signal pathways overactive in absence of mitogenic stimulation. Similarly, mutations that cause an overexpression of Myc promote excessive cell growth, which in turn promotes the development of cancer.

Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* regulate the components of the Ras-dependent or Myc pathway to make sure it is not overactive.

10

The compound or its derivatives reactivate the abnormal cell checkpoint mechanism. Inside the cell, there is a checkpoint mechanism which detects abnormal mitogenic stimulation and causes abnormally overactive cells to go into apoptosis.

15

However this mechanism is not active in cancer cells due to mutations in the genes that encode essential components of the checkpoint responses. If the mutation happens in the checkpoint mechanism, the cancer cell will growth and divide endlessly.

20

Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* reactivate the checkpoint mechanism to stop the cancer cell growth.

25

The compound or its derivatives affect the extracellular growth signaling pathways. The extracellular growth factors that stimulate cell growth are bound to receptors on the cell surface and activate intracellular signaling pathways. It activates the enzyme PI3-kinase, which promotes protein synthesis, at least partly through the activation of eif4e and phosphorylated s6 kinase, resulting in increased mRNA translation and then a stimulation of cell growth.

30

Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* regulate the components or receptor relate to extracellular growth. It binds the receptor of ovarian cancer

35

cells so as to stop the cancer cell growth.

Compounds or compositions derived from the plant *Xanthoceras*
Sorbifolia regulate the components relating to Ras and MAP
5 Kinase, which ceases ovarian cancer cell growth.

The compound or its derivatives affect the intracellular
mechanism. Cell division is also controlled by an intracellular
mechanism that can limit cell proliferation. In normal cells,
10 the Myc protein acts in the nucleus as a signal for cell
proliferation. Large quantities of Myc can cause the cell to
proliferate in excess and form a tumor.

Compounds or compositions derived from the plant *Xanthoceras*
15 *Sorbifolia* regulate the components or receptor of the Myc cell's
proliferation to stop the tumor cells from dividing.

The compound or its derivatives affect the TGF-alpha signaling
pathway. TGF-alpha is produced by keratinocytes, macrophages,
20 hepatocytes, and platelets. Its synthesis is stimulated by the
infection by viruses. TGF-Alpha induces the long term
proliferation of murine and chicken immature hematopoietic
progenitor cell such as BFU-E without causing differentiation.
It also induces the terminal differentiation of BFU-E cell into
25 erythrocytes. TGF-Alpha stimulates the proliferation of
cultured endothelial cells. It plays an importance role in the
vascularisation of tumor tissues.

Compounds or compositions derived from the plant *Xanthoceras*
30 *Sorbifolia* regulate the components or receptor of TGF-alpha to
suppress ovarian cancer and bladder cancer cell growth.

The compound or its deviative compounds affect the TGF-beta
signaling pathway. TGF-beta regulates growth and proliferation
35 of cells, blocking growth of many cell types. There are two

TGF-beta receptors: Type 1 and Type 2. They are serine-threonine kinases that signal through the SMAD (Protein named after the first two identified, Sma in *C. elegans* and Mad in *Drosophila*) family of transcriptional regulators. The TGF-beta pathway and mutation in SMADs are associated with cancer in humans.

Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* regulate the components or receptor of TGF-beta to suppress the ovarian cancer and bladder cancer cell growth.

The compound or its derivatives reactivate the cell functions which are damaged by DNA viruses. DNA tumor viruses cause cancer by interfering with cell cycle control Rb protein and the p53 protein. Mutation in p53 gene will allow cancer cells to survive and proliferate despite DNA damage. The papillomavirus uses the proteins E6 and E7 to sequence the p53 and Rb respectively. This action activates mutated cells, allowing them to survive and then divide and accumulate. The accumulation of damaged cells can lead to cancer.

Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* regulate the proteins E6 and E7 and release the proteins Rb and p53, which will prevent abnormal cells from dividing. It also regulates or reacts with the protein, causing the cancer cells to die.

The compound or its derivatives affect the p53 signaling pathway. p53 helps multi-cellular organisms cope safely with DNA damage and other stressful cellular events, stopping cell proliferation in circumstances where it would be dangerous. Cancer cells tend to contain large quantities of mutant p53 protein, suggesting that the genetic accidents they undergo or the stresses of growth in an inappropriate environment created the signals that normally activate the p53 protein. Thus, the loss of p53 activity can be extremely dangerous in relation to

cancer because it allows mutant cells to continue through the cell cycle. It also allows them to escape apoptosis. So, if their DNA is damaged, some cells will die but the cells which survive will carry on dividing without pausing to repair the damage.

5 This may cause the cells to die, or they could survive and proliferate with a corrupted genome, which could lead to loss of both tumor suppressor genes and the activation of oncogenes, for example by gene amplification. Gene amplification could enable cells to develop resistance against therapeutic drugs.

10

Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* regulate the components and receptor of the p53 pathway, which stops the cancer cells from dividing.

15 The compound or its derivatives affect the cell suicide signaling pathway. All cells with a nucleus contain various inactive procaspases, awaiting a signal before destroying the cell. Each suicide protease is made as an inactive proenzyme called procaspase. It is usually activated by proteolytic
20 cleavage by another member of the caspase family. Two of the cleaved fragments come together to form the active part of the caspase, and the active enzyme is thought to be a tetramer of two of these two parts. Each activated caspase molecule can cleave many procaspase molecules, which in turn activates more
25 molecules. Through a chain reaction or cascade, this leads to the explosive action of a large number of procaspase molecules. Then, some of the activated procaspases cleave a number of key proteins in the cell, including specific cytosolic proteins and nuclear-lamins leading to the controlled death of the cell.

30

Activating the death receptor on the outside of the cell can also trigger inactive procaspases. For example, killer lymphocytes, can cause apoptosis by producing the protein Fas on the surface of the targeted cell. These clusters of Fas
35 protein then recruit intracellular adaptor proteins that bind

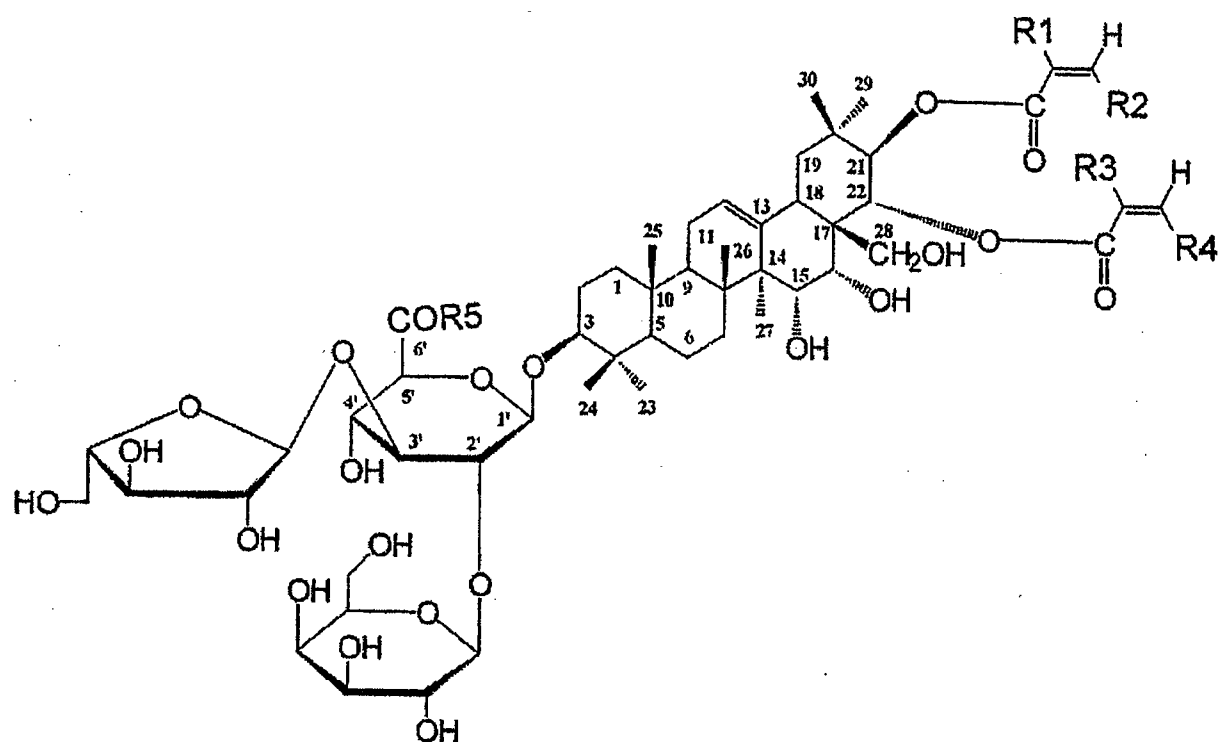
and aggregate procaspase-8 molecules. These then cleave and activate one another. The activated caspase-8 molecules then activate downstream procaspases to induce apoptosis.

- 5 However in cancer cells, the signal to destroy the cell is blocked, due to gene mutation. This means that the cancer cells continue to divide, thereby causing a tumor.

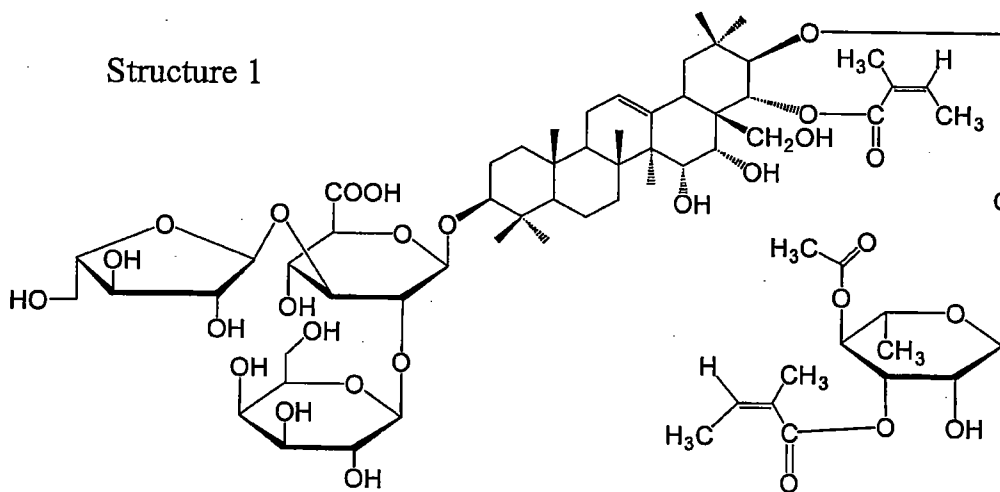
- 10 Compounds or compositions derived from the plant *Xanthoceras Sorbifolia* unblock the suicide signals, allowing cancer cells to destroy themselves.

- 15 This invention provides a method for inhibiting tumor cell growth comprising contacting an amount of the compound, wherein R1, R2, R3, R4 are short aliphatic chain and R5 contains an oxyl group; and a pharmaceutically acceptable carrier effective to inhibit growth of said tumor cells.

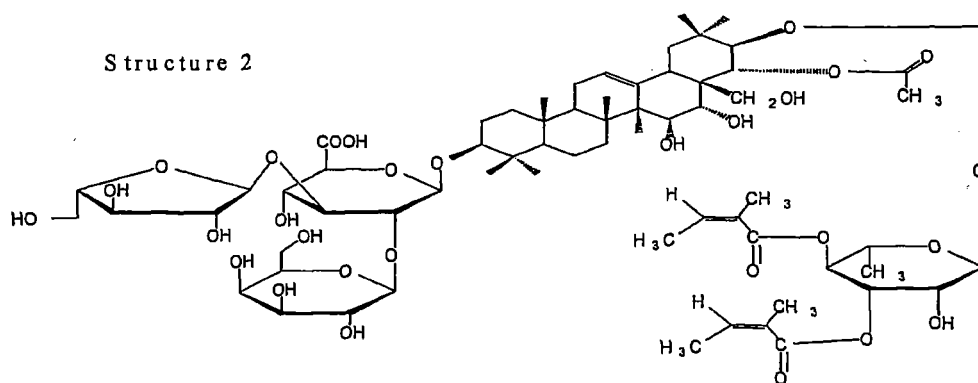
- 20 This invention provides the above method, wherein R1=R2=R3=R4=CH3 and R5=OH.



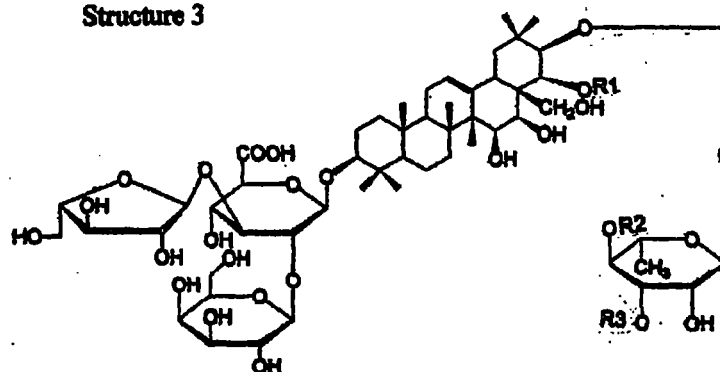
Structure 1



Structure 2



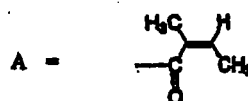
Structure 3



Where R1 = A or B or C

R2 = A or B or C

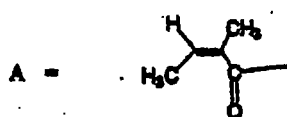
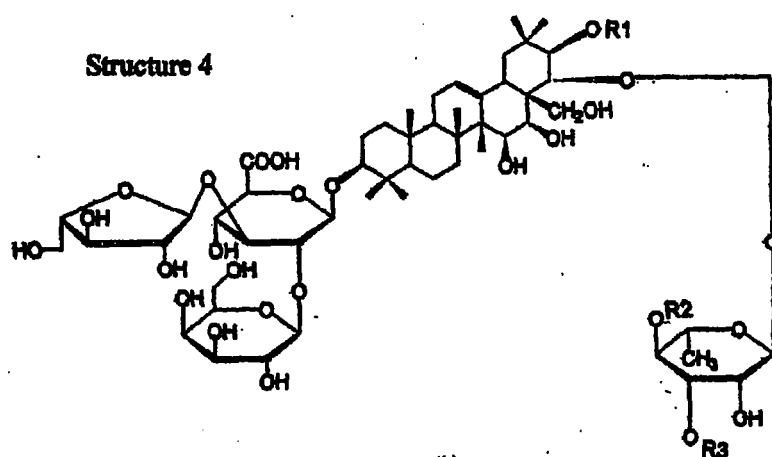
R3 = A or B or C



	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C



Where R1 = A or B or C

R2 = A or B or C

R3 = A or B or C



C = H

	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

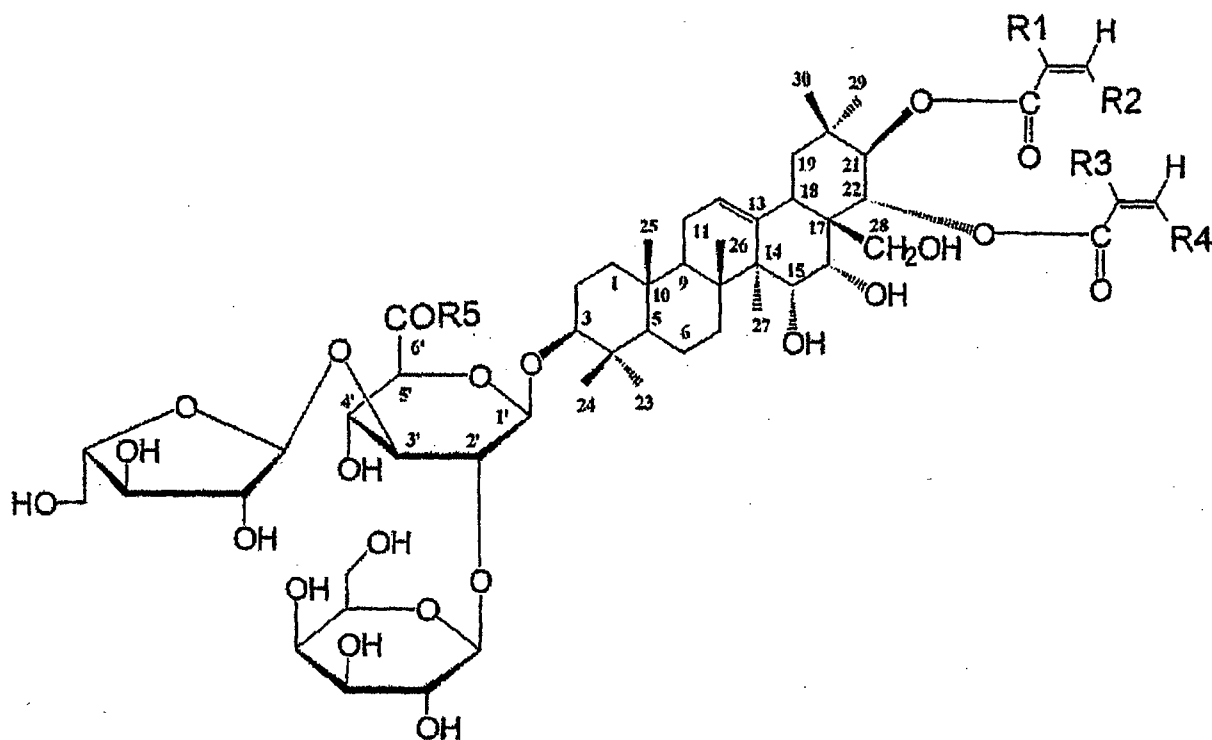
This invention provides a method for inhibiting tumor cell growth comprising contacting an amount of the compound comprising: a sugar; a terepene or Sapogenin; side chain at Carbon 21 and 22 or Angeloyl groups, operatively linked form the compound; and a pharmaceutically acceptable carrier.

This invention provides a method for inhibiting tumor cell growth in a subject comprising administering to the subject,

5 wherein R1, R2, R3, R4 are short aliphatic chain and R5 contains an oxyl group; effective to inhibit growth of said tumor cells and a pharmaceutically acceptable carrier.

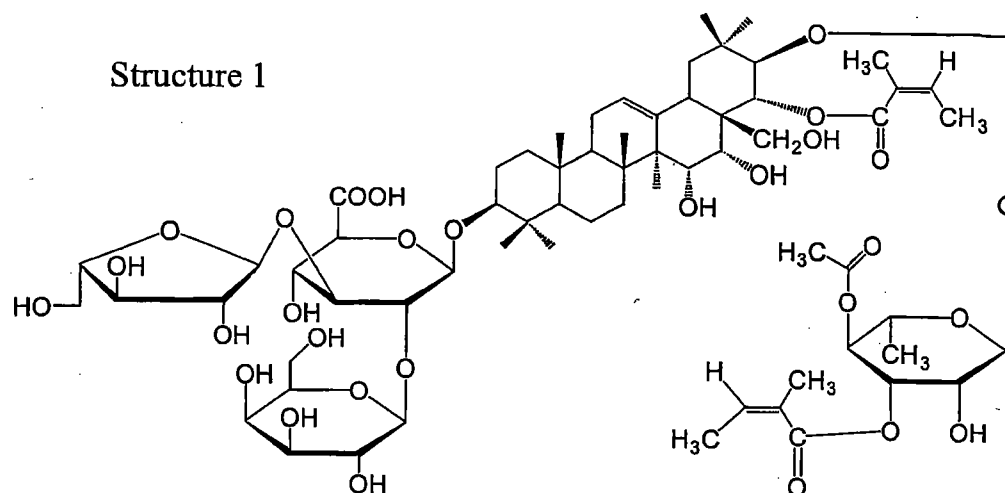
This invention provides a method of claim 27, wherein R1=R2=R3=R4=CH3 and R5 contains an oxyl bond.

10

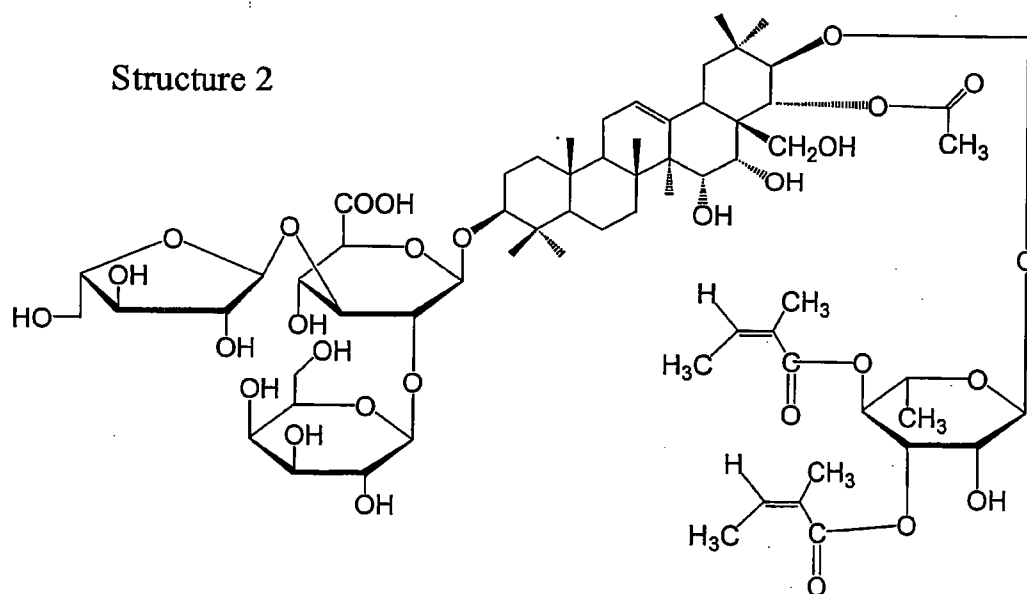


15

Structure 1



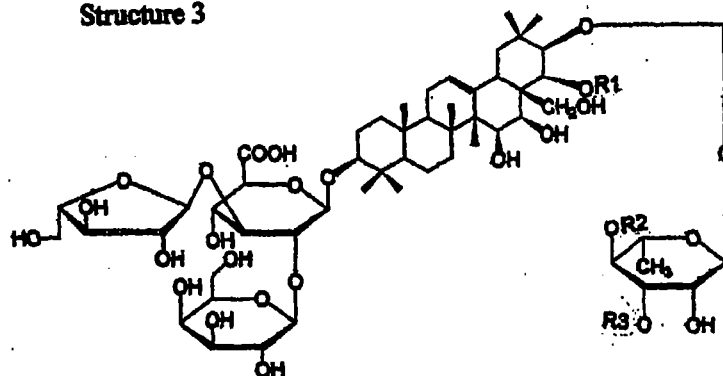
Structure 2



5

10

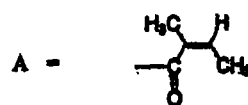
Structure 3



Where R1 = A or B or C

R2 = A or B or C

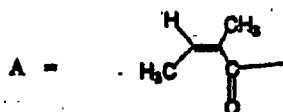
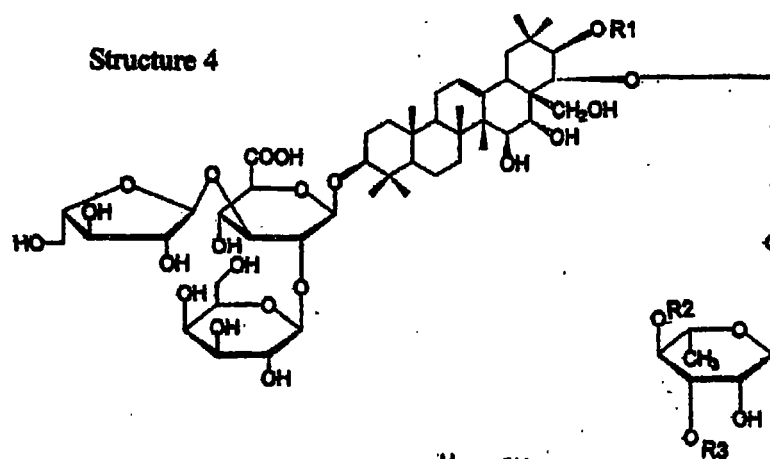
R3 = A or B or C



	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

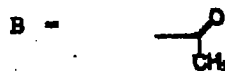
19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C



Where R1 = A or B or C

R2 = A or B or C

R3 = A or B or C



	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

This invention provides a method for inhibiting tumor cell growth in a subject comprising administering to the subject a compound comprising: a sugar; a terepene or Sapogenin; side chain at Carbon 21 and 22 or Angeloyl groups, operatively linked
5 form the compound; and a pharmaceutically acceptable carrier.

This invention will be better understood from the examples which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the
5 claims which follow thereafter.

EXPERIMENTAL DETAILS**Experiment 1:**

Effects of X and Y on improving learning on normal aging mice

X and Y are different Wenguanguo extracts

Aging male mice at 16 months of age weighing 35-55 gm were trained in a SMG-2 filled with water 11cm deep (25-26°C). The SMG-2 had a start point, 4 blind terminals, the escape platform and their routes. The mice were trained to find the escape platform, and the escape latencies from the water and error frequencies were recorded. After training 3 times, the mice which escaped from the water in 2 minutes were selected for the test. The selected aging mice were divided into 9 groups of 11: 1) control: receiving normal saline (NS); 2) Positive control: receiving Xi-en-kai 0.9g/kg; 3) X-I group: receiving 100mg/kg; 4) X-II group: receiving 200mg/kg; 5) X-III group: receiving 400mg/kg; 6) Y-I group: receiving 125mg/kg; 7) Y-II group: receiving 250mg/kg; 8) Y-III group: receiving 500mg/kg and 9) model group.

All the drugs were received through oral administration, 20ml/kg, 3, 6 and 9 days before the water maze test. The escape latencies (EL) from the water and error frequencies were recorded. All data were analyzed with t-test.

1.1. After 3 days of administration of X and Y, the escape latency in the water maze by the group 9 mice receiving 500 mg/kg of Y decreased significantly compared with the control ($P < 0.05$). The other dosage treatments showed improvement too but not significant ones.

Table 1.1. The learning effects of Plant extract administration of aging mice for 3 days

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
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control	2.12±2.27	3.62±1.63	1.37±2.41	2.68±2.52	9.81±3.42	64.12±24.8
positive	1.09±1.57	2.0±0.89	2.0±1.26	2.0±2.64	7.09±2.30	39.45±16.32
X I	1.18±1.4	3.36±2.65	2.27±2.14	0.81±1.07	7.63±4.47	51.72±17.23
X II	2.2±1.61	2.5±1.51	2.0±1.69	2.8±1.03	9.5±4.03	50.3±20.84
X III	1.45±2.33	2.72±2.64	2.09±2.11	1.81±1.94	8.09±4.67	46.91±19.18
Y I	1.36±1.91	3.0±1.94	3.45±3.2	1.09±1.44	8.18±3.78	46.36±22.33
Y II	1.63±1.80	3.81±1.94	2.36±1.12	2.36±1.50	10.18±3.02	48.36±20.61
Y III	2.18±3.34	1.63±1.21	1.54±1.29	1.81±1.40	7.18±4.30	41.45±16.48*

P<0.05*

1.2. After 6 days of administration of X and Y, the error frequency in the water maze in the mice with all dosage treatments decreased significantly (P<0.05, P<0.01). The escape latency in the water maze by the group 9 mice receiving 500 mg/kg of Y decreased significantly (P<0.05).

10 **Table 1.2. The learning effects of Plant extract administration of aging mice for 6 days**

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
Control	2.74±2.98	4.0±2.67	3.26±3.02	3.0±2.67	12.9±4.20	60.58±24.6
positive	1.64±2.06	3.73±2.05	1.18±1.47	2.0±1.90	8.55±4.61*	38.64±13.68
X □	1.7±1.49	3.1±2.02	1.8±2.10	1.9±1.66	8.5±1.65*	47.7±11.13
X □	1.18±1.66	3.5±2.02	2.0±1.73	1.73±2.05	8.45±3.14*	49.0±13.29
X □	1.09±1.30	3.55±2.07	1.91±1.58	1.55±1.44	8.0±2.32*	46.36±13.31
Y □	1.0±1.67	3.09±1.58	2.64±1.8	1.54±2.02	8.36±3.07*	57.27±19.88
Y □	1.36±1.50	3.36±2.06	2.0±1.67	1.18±1.17	7.91±3.05**	47.55±22.93
Y □	1.2±1.14	4.1±1.79	2.5±2.84	1.4±1.90	9.0±3.23*	39.9±8.56*

P<0.05* P<0.01**

1.3. After 9 days of administration of X and Y, the error frequency in the water maze in the mice with all dosage treatments decreased significantly (P<0.05, P<0.01). The escape latency in the water maze by the group 9 mice receiving 500 mg/kg of Y decreased significantly (P<0.05).

20 **Table 1.3. The learning effects of Plant extract administration of aging mice for 9 days**

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
-------	---------------------	---------------------	---------------------	---------------------	-----------------	---------

Control	2.36±1.65	2.64±1.69	2.86±2.54	1.5±2.18	9.71±3.52	59.71±29.42
positive	1.64±1.80	2.18±1.54	1.55±1.37	1.64±2.20	7.0±2.19*	33.36±10.87
X □	1.1±1.29	2.5±2.37	1.9±0.74	1.0±1.15	6.5±3.27*	40.8±20.4
X □	1.18±1.17	2.18±1.60	1.36±1.36	2.18±1.25	6.91±3.27*	42.73±15.82
X □	1.0±1.25	1.9±1.79	1.3±1.25	2.2±1.16	6.4±2.84*	35.1±11.76*
Y □	1.82±1.33	1.64±1.69	1.82±1.33	1.82±1.47	7.09±2.47*	42.09±20.93
Y □	1.2±1.32	1.9±1.37	2.6±1.58	1.2±1.32	7.1±1.52*	34.4±13.47*
Y □	0.8±1.03	2.5±1.43	1.8±1.40	2.0±1.70	7.0±1.41*	31.9±9.87**

P<0.05* P<0.01**

The results indicated that the extracts X and Y had positive effects on improving acquisition and retention of the tested aging mice. In addition, the effects increased with the period of receiving the extracts of X and Y prolonged.

Experiment 2:

Effects of X and Y on improving impairment induced by pentobarbital sodium in water maze

2.1 After 10 days of administration of the extracts X and Y, the administrated mice were injected with pentobarbital sodium to induce amnesia.

After 1 day administration of pentobarbital sodium, the results of water maze learning showed that the time spent searching the terminal platform in the water maze by the mice receiving 100 mg/kg of X, and 125mg, 250mg/kg and 500mg/kg of Y decreased significantly (P<0.05).

Error frequency made in the water maze by the mice receiving 500mg/kg of Y decreased significantly (P<0.05).

Table 2.1. The result of water maze learning of 1st-day-injected pentobarbital

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
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control	0.92±0.76	1.46±0.97	1.85±1.07	1.23±0.73	5.38±2.33	63.0±25.31
positive	0.69±0.70	1.3±0.95	1.1±0.74	1.1±0.74	4.1±1.85	36.5±15.76**
X I	0.5±0.53	1.7±0.82	1.2±0.92	0.9±0.32	4.2±1.62	42.2±18.83*
X II	0.9±0.88	1.4±0.70	1.6±1.35	1.1±0.88	5.0±2.49	53.8±16.10
X III	0.9±0.74	1.7±0.82	1.8±0.42	0.9±0.57	5.4±1.58	58.1±16.11
Y I	1.0±0.89	1.36±0.81	1.27±1.01	0.73±0.65	4.09±2.02	42.73±16.17*
Y II	0.9±0.74	1.7±0.82*	1.0±0.82*	0.6±0.70*	4.2±1.87	38.4±15.19**
Y III	0.6±0.70	0.8±0.63	1.4±1.35	0.8±0.63	3.6±1.26*	38.5±13.81**

P<0.05* P<0.01**

2.2. After two days of injected of pentobarbital sodium, the time spent searching the terminal platform and the error frequency made in the water maze by all groups of mice receiving X and Y decreased significantly (P<0.05).

Table 2.2. The result of water maze learning of 2nd-day-injected pentobarbital

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
control	1.36±0.50	1.86±0.53	1.29±1.07	1.14±0.66	5.64±1.91	59.5±34.95
positive	0.8±0.63	1.8±0.42	1.1±0.74	0.6±0.70	4.3±0.95*	34.7±11.45*
X I	0.9±0.57*	1.0±0.67**	1.2±0.79	0.8±0.63	3.9±1.73*	34.5±12.67*
X II	0.8±0.79*	1.5±0.71	1.1±0.88	0.5±0.53*	3.8±1.40*	35.9±12.70*
X III	1.0±1.05	1.3±0.48*	1.3±0.82	0.5±0.53*	4.1±1.79*	36.1±11.10*
Y I	1.09±0.94	1.45±0.52	0.91±0.83	0.73±0.65	4.18±1.08*	36.64±14.38*
Y II	0.9±0.57*	1.3±0.82	1.1±0.88	0.8±0.63	4.1±1.45*	35.5±14.27*
Y III	0.8±0.63*	0.9±0.74**	0.9±0.57	0.9±0.57	3.4±1.43**	32.1±13.12*

P<0.05* P<0.01**

2.3. After three days of administration of pentobarbital sodium, the time spent searching the terminal platform in the water maze by all groups of mice receiving X and Y decreased significantly (P<0.05). The error frequency made in the water maze by the mice receiving 250mg/kg and 500mg/kg of Y decreased significantly (P<0.05).

Table 2.3. The result of water maze learning of 3rd-day-injected pentobarbital

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
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control	0.85±0.99	1.0±0.58	1.46±1.05	0.62±0.65	3.92±1.75	48.92±23.49
positive	0.4±0.52	0.7±0.48	0.9±0.74	0.6±0.70	2.6±0.97*	28.4±13.78*
X I	0.6±0.70	0.8±0.63	0.6±0.70*	0.8±0.92	2.8±1.69	28.0±17.10*
X II	0.4±0.52	1.0±0.47	1.0±0.82	0.6±0.84	3.0±1.25	32.0±12.36*
X III	0.5±0.71	0.8±0.63	1.0±0.82	0.9±0.74	3.2±1.23	31.8±12.48*
Y I	0.5±0.53	0.7±0.48	1.0±0.67	0.6±0.52	2.8±0.63	31.0±9.66*
Y II	0.5±0.53	0.6±0.52	0.8±0.42	0.6±0.52	2.5±0.53*	29.0±7.80*
Y III	0.3±0.48	1.0±0.47	0.9±0.57	0.4±0.52	2.6±0.84*	30.2±7.54*

P<0.05*

The results indicated that the extracts X and Y had distinct positive effects on improving spatial learning and retention impairment induced by pentobarbital sodium.

Experiment 3:

Effects of X and Y on improving impairment induced by Scopolamine hydrobromide in passive avoidance

ICR mice weighing 16-20 gm were trained in a STT-2. A mouse was placed on the platform and the SDL were recorded. When the mouse stepped down and all four feet were on the grid, it received electric shock (36 V) immediately, and the EL was recorded. The mice with SDL and EL within 2-60 seconds were selected for the test. The selected aging mice were divided into 9 groups. Each group had 5 male and 5 female mice. All the drugs were received with oral administration, 20ml/kg, 3, 6 and 9 days before the platform training. The SDL, EL and error frequency (the times of receiving electric shocks) made in 5 minutes were recorded. After 10 days of administration all the groups of mice received Scopolamine hydrobromide by injection, 3mg/kg. After 30 minutes of administration of Scopolamine hydrobromide the mice were trained on the platform and the training was repeated the next day. The performances of the mice in the training were recorded. The SDL, EL and error frequency were recorded. All data were analyzed with t-test.

3.1. After 9 days of administration of X and Y, the results of passive avoidance in aging mice showed that the EL and the error

frequency made by the mice received the X and Y in all doses reduced.

Table 3.1. Passive avoidance in aging mice after 9 days administration

group	EL (sec)	SDL (min)	error frequency (in 5min)
control	6.8±5.93	131.5±106.50	2.5±1.47
positive	5.3±3.74	148.6±81.26	1.7±1.25
X□I	7.0±5.46	143.3±19.77	1.8±0.92
X□II	6.2±5.71	141.1±109.89	2.0±1.25
X□III	4.9±3.70	145.7±107.0	1.9±1.52
Y□I	5.1±3.95	153.0±123.52	1.6±1.33
Y□II	3.9±2.77	162.7±108.92	1.8±1.48
Y□III	5.7±3.12	159.4±83.20	1.9±0.93

3.2. After 10 days of administration of the X and Y, the tested mice were administrated with scopolamine. The results of passive avoidance in aging mice showed that the error frequency made in the passive avoidance by the mice receiving 400mg/kg of X, 250mg/kg and 500mg/kg of Y reduced significantly ($P<0.05$, $P<0.01$).

Table 3.2. Effects of plant extract X and Y on improving impairment induced by Scopolamine

group	EL (sec)	error frequency (5min)
control	3.4±3.03	0.9±1.29
model	4.4±4.09	1.6±1.35
positive	3.1±1.20	0.4±0.52*
X□I	3.1±2.08	0.8±1.03
X□II	3.7±3.06	0.9±1.52
X□III	2.8±1.48	0.3±0.48**
Y□I	3.2±2.49	0.7±1.06
Y□II	2.5±0.97	0.5±0.71*
Y□III	2.5±0.71	0.5±0.71*

* $p<0.05$ ** $p<0.01$

The results of passive avoidance test impaired mice by scopolamine showed that the error frequency made by the mice receiving X and Y in all doses reduced significantly ($P<0.05$). The SDL prolonged significantly in mice receiving 250 mg/kg of Y.

The results indicated that the extracts X and Y had distinct positive effects on improving learning and retention impairment induced by scopolamine.

5 **Table 3.3 Effects of plant extract X and Y on improving impairment induced by Scopolamine.**

group	SDL(sec)	error frequency(5 min)
control	230.4±96.61	0.7±1.06
model	216.2±100.77	1.5±1.35
positive	286.0±34.38*	0.4±0.70*
X□I	245.7±114.48	0.4±0.84*
X□II	260.4±87.14	0.4±0.84*
X□III	266.8±65.64	0.5±0.71*
Y□I	252.7±101.11	0.4±0.84*
Y□II	285.8±29.21*	0.4±0.70*
Y□III	277.4±47.62	0.4±0.70*

*p<0.05

10 The results indicated that the extracts X and Y had distinct positive effects on improving learning and retention impairment induced by scopolamine hydrobromide.

Experiment 4:

15 **Effects of X and Y on improving impairment induced by in HaCO₂ water maze**

ICR male mice weighing 16-19 gm were trained in a SMG-2 filled with water 11cm deep (25-26°C). The SMG-2 has a start point, 4 blind terminals, the escape platform and their routes. The mice were trained to find the escape platform, and the escape latencies from the water and error frequencies were recorded. After training, the mice which escaped from the water within 1 minute were selected for the test. The selected aging mice were divided into 9 groups of 11 mice. All the drugs were received with oral administration, 20ml/kg, 3, 6 and 9 days before the water maze test. After 10 days of administration all the groups of mice were received with HaCO₂ by injection, 120 mg/kg. After 24 hours of administration of HaCO₂ the mice were trained to find the escape platform, and the escape latencies from the water and error frequencies made in 2 minutes

were recorded. The administration of pentobarbital sodium continued for 3 days and the performances of the mice in water maze test were recorded. The escape latencies (EL) from the water and errors frequencies were recorded. All data were analyzed with t-test.

4.1. After 3 days of administration of X and Y the escape latency from the water maze and error frequency by the mice receiving X and Y reduced, although not significantly.

Table 4.1. After 3 days of administration of X and Y on learning in mice in water maze

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
control	1.05±0.49	1.82±0.66	1.09±0.75	0.86±0.47	4.82±0.96	48.27±21.47
positive	0.91±0.30	1.27±0.65	0.82±0.60	0.64±0.50	3.64±0.92**	36.27±11.83
X □ I	1.36±0.81	1.73±0.79	1.09±0.83	0.55±0.52	4.73±2.05	37.82±14.24
X □ II	0.91±0.30	1.82±0.40	1.09±0.94	1.00±0.89	4.91±1.51	36.46±11.97
X □ III	1.09±0.54	1.45±0.52	0.91±0.70	0.45±0.52	3.91±0.70	36.46±11.78
Y □ I	1.55±0.52	1.82±0.40	1.0±0.89	0.45±0.69	4.82±1.33	41.46±16.37
Y □ II	0.91±0.30	1.18±0.60	1.27±1.10	0.73±0.79	4.09±2.21	36.82±20.61
Y □ III	0.91±0.30	1.55±0.82	0.45±0.52	0.82±0.40	3.73±1.27	37.55±13.85

*P<0.05 **P<0.01

4.2. After 6 days of administration of X and Y the escape latency from the water maze by the mice receiving 400mg/kg of X and 500mg/kg of Y reduced significantly compared with the control (P<0.01).

Table 4.2 After 6 days of administration of X and Y on learning in mice in water maze

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
control	0.95±0.38	1.09±0.43	0.77±0.61	0.91±0.53	3.82±0.80	42.96±13.48
positive	0.73±0.65	1.09±0.30	0.55±0.52	0.82±0.40	3.18±0.75*	32.91±7.15*
X □ I	0.73±0.65	1.27±0.47	0.73±0.65	0.73±0.65	3.45±1.21	37.18±7.65
X □ II	0.91±0.30	1.00±0.45	0.64±0.92	0.91±0.54	3.45±1.21	37.73±13.26
X □ III	0.91±0.30	1.09±0.54	0.91±0.83	0.82±0.75	3.73±1.19	31.09±8.15**
Y □ I	0.91±0.30	1.0±0.45	0.82±0.40	0.55±0.52	3.36±0.67	35.82±9.93
Y □ II	0.91±0.54	1.09±0.54	1.00±0.77	0.64±0.67	3.64±1.12	35.09±12.13
Y □ III	0.82±0.40	1.09±0.54	0.82±0.60	0.55±0.52	3.27±1.10	31.73±8.36**

*P<0.05 **P<0.01

4.3. After 9 days of administration of X and Y, the escape latency from the water maze by the mice receiving 250mg/kg of X, 250mg and 500mg/kg of Y reduced significantly compared with the control ($P < 0.05$).

5

Tablet 4.3. After 9 days of administration of X and Y on learning in mice in water maze

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
control	0.59±0.73	1.14±0.64	0.55±0.67	1.0±0.76	3.27±1.32	39.27±15.52
positive	0.55±0.52	1.00±0.00	0.27±0.65	0.91±0.54	2.73±0.65	27.64±6.96*
X □ I	0.45±0.52	1.27±0.47	0.73±0.65	0.55±0.52	3.00±0.77	33.55±9.59
X □ II	0.45±0.52	0.91±0.70	0.55±0.69	0.82±0.89	2.73±0.90	28.00±9.53*
X □ III	0.45±0.52	1.09±0.70	0.82±0.75	0.45±0.52	2.82±1.25	29.45±8.49
Y □ I	0.91±0.70	0.91±0.54	0.45±0.52	0.55±0.52	2.82±0.98	32.00±9.49
Y □ II	0.64±0.50	0.82±0.75	0.64±0.67	0.82±0.60	2.91±1.30	26.36±9.82*
Y □ III	0.73±1.01	0.91±0.30	0.45±0.69	0.55±0.69	2.64±1.12	28.09±9.26*

* $P < 0.05$

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4.4. After 10 days of administration of X and Y, the error frequency made in the water maze by the mice receiving 250mg and 500mg/kg of Y, the escape latency from the water maze by the mice receiving 500mg/kg of Y reduced significantly compared with the control ($P < 0.05$, $P < 0.01$).

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Table 4.4. After 10 days of administration of X and Y on learning in mice in water maze

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
control	0.64±0.73	1.14±0.56	0.64±0.66	0.91±0.68	3.32±0.95	36.73±13.02
positive	0.45±0.69	0.82±0.40	0.55±0.52	0.82±0.40	2.64±0.81*	29.0±10.10
X □ I	0.36±0.67	0.91±0.70	0.91±0.83	0.64±0.81	2.82±1.08	33.09±13.96
X □ II	0.73±0.79	0.82±0.40	0.55±0.69	0.73±0.47	2.82±0.75	28.91±13.34
X □ III	0.91±0.70	0.91±0.54	0.73±0.65	0.36±0.67	2.91±0.94	32.45±13.57
Y □ I	0.73±0.79	0.91±0.30	0.36±0.50	0.73±0.65	2.73±0.90	29.55±13.87
Y □ II	0.55±0.52	0.64±0.50	0.45±0.52	0.64±0.67	2.27±0.79**	30.36±12.30
Y □ III	0.45±0.69	1.09±0.54	0.27±0.65	0.55±0.52	2.36±1.21*	25.64±11.02*

* $P < 0.05$ ** $P < 0.01$,

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The results indicated that the extracts X and Y had distinct positive effects on improving the learning and retention in mice in a water maze. In addition, the effects increased with the period of receiving the extracts of X and Y prolonged.

4.5. After 10 days of receiving X and Y, the mice were administrated with Na NO₂ after the test. The results of treatment with X and Y to prevent impairments induced by Na NO₂ in water maze learning in aging mice showed that error frequency made by the mice receiving 100mg/kg and 200mg/kg of X, and Y of all doses reduced significantly ($P < 0.05$) (Table 4.5). It indicated the extracts X and Y had distinct positive effects on preventing the impairments induced by Na NO₂.

Table 4.5. The effects of extract X and Y on preventing the impairments induced by Na NO₂

Group	Blind Terminal 1	Blind Terminal 2	Blind Terminal 3	Blind Terminal 4	error frequency	EL(sec)
Control	0.27±0.47	0.91±0.54	0.55±0.52	1.09±0.54	2.82±0.75	30.91±12.36
Model	1.18±0.75	0.91±0.30	0.45±0.52	0.73±0.65	3.27±1.01	36.45±16.89
Positive	0.45±0.52	0.91±0.30	0.55±0.52	0.45±0.52	2.36±0.81*	32.00±15.83
X I	0.55±0.52	0.82±0.60	0.18±0.40	0.82±0.60	2.36±0.81*	29.09±13.80
X II	0.18±0.40	0.82±0.60	0.55±0.52	0.82±0.75	2.36±0.92*	25.82±10.82
X III	0.45±0.52	0.64±0.50	0.82±0.40	0.91±0.70	2.82±1.33	31.09±11.76
Y I	0.27±0.47	0.91±0.30	0.36±0.50	0.73±0.47	2.27±0.79*	27.00±10.73
Y II	0.45±0.52	0.64±0.50	0.36±0.50	0.91±0.30	2.36±0.81*	25.82±11.43
Y III	0.64±0.50	0.82±0.40	0.36±0.50	0.64±0.50	2.45±0.82*	25.09±9.67

* $P < 0.05$ ** $P < 0.01$

Experiment 5:

The effects of Wenguanguo (*Xanthoceras sorbifolia*) extract on urination in mice

X and Y are different extract of Wenguanguo; Where X is a kind of Wenguanguo (*Xanthoceras sorbifolia*) extract; and Where Y is a kind of Wenguanguo (*Xanthoceras sorbifolia*) extract.

Methods of Experiment

One hundred twelve male ICR mice weighing 18-22 gm were divided into 8 groups of 14: 1, control: receiving normal saline (NS); 2, DCT group: receiving DCT 33.4 mg/kg; 3, X-I group: receiving 100mg/kg; 4, X-II group: receiving 200mg/kg; 5, X-III receiving 400 mg/kg; 6, Y-I group: receiving 125mg/kg; 7, Y-II group: receiving 250mg/kg and 8, Y-III group: receiving 500 mg/kg.

All the drugs were received with oral administration, 20ml/kg, once a day for 3 days. After the last administration, the mouse was placed on a filter paper. The filter paper was on the bottom of a 500 ml beak. The quantity of urine was measured at 30, 60, 120, 180, 240, 300, and 360 minutes by weighing the filter paper with the electronic analytical scale. All data were analysed with t-test.

Results

After 3 days of administration of X and Y the quantity of urine discharged at 30 minute by the mice receiving 400 mg/kg of X decreased significantly ($P < 0.01$) compared with the mice receiving normal saline. The quantity of urine discharged at 60 minute by the mice receiving 600 mg/kg of Y decreased significantly compared with the mice receiving normal saline ($P < 0.01$).

The quantity of urine discharged at 180 minutes by the mice receiving 200 mg/kg of X, 125 mg and 500mg/kg of Y decreased significantly compared with the mice receiving normal saline ($P < 0.01$) (Table 5.1).

Table 5.1. Effects of the extract X and Y on the quantity of urine in mice after 3 days of administration of X and Y

Group	30min	60min	120min
Control	0.267±0.105	0.367±0.162	0.382±0.109
Positive	0.348±0.06*	0.471±0.169**	0.574±0.249
X I	0.304±0.072	0.274±0.076	0.323±0.173
X II	0.341±0.107	0.323±0.102	0.404±0.138
X III	0.155±0.056**	0.200±0.140 **	0.455±0.211
Y I	0.216±0.130	0.309±0.093	0.341±0.061
Y II	0.278±0.063	0.278±0.119	0.437±0.112
Y III	0.227±0.058	0.235±0.035**	0.425±0.133

Compared with the control group: $P < 0.05^*$ $P < 0.01^{**}$

Table 5.1. Effects of the extract X and Y on the quantity of

urine in mice after 3 days of administration of X and Y
(continued)

Group	180min	240min	300min	360min
Control	0.191±0.080	0.161±0.083	0.116±0.06	0.103±0.057
Positive	0.272±0.131	0.182±0.096	0.110±0.051	0.085±0.031
X I	0.184±0.105	0.154±0.093	0.124±0.091	0.102±0.064
X II	0.336±0.103**	0.163±0.10	0.107±0.076	0.106±0.075
X III	0.207±0.112	0.204±0.088	0.150±0.066	0.116±0.077
Y I	0.367±0.104**	0.171±0.085	0.173±0.068*	0.093±0.053
Y II	0.275±0.206	0.145±0.029	0.109±0.036	0.106±0.045
Y III	0.319±0.086**	0.264±0.114**	0.152±0.084	0.135±0.051

Compared with the control P<0.05* and P<0.01**

- 5 The results indicated that the extract X and Y can regulate the quantity of urine after 3 days of administration of X and Y.

After 5 days of administration of X and Y, the quantity of urine discharged at 30 minute by the mice receiving 400 mg/kg of X and 500mg/kg of Y decreased, but not significantly compared with the mice receiving normal saline. The quantity of urine discharged at 4 hour by the mice receiving 400 mg/kg of X and Y in all doses increased significantly compared with the mice receiving normal saline (P<0.05, P<0.01) (Table 5.2).

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Table 5.2. Effects of the extract X and Y on the quantity of urine in mice after 5 days of administration of X and Y

Group	30min	60min	120min
Control	0.327±0.148	0.330±0.194	0.291±0.146
Positive	0.524±0.206**	0.478±0.185*	0.472±0.292*
X I	0.382±0.138	0.251±0.071	0.265±0.172
X II	0.348±0.144	0.324±0.113	0.277±0.131
X III	0.245±0.167	0.236±0.129	0.251±0.142
Y I	0.331±0.098	0.340±0.133	0.291±0.081
Y II	0.357±0.130	0.290±0.145	0.327±0.157
Y III	0.230±0.121	0.307±0.082	0.363±0.100

Compared with the control P<0.05* and P<0.01**

- 20 Table 5.2. Effects of the extract X and Y on the quantity of

urine in mice after 5 days of administration of X and Y
(continued)

Group	180min	240min	300min	360min
Control	0.186±0.086	0.117±0.069	0.105±0.06	0.104±0.08
Positive	0.214±0.151	0.110±0.045	0.126±0.056	0.112±0.065
X I	0.188±0.097	0.175±0.088	0.177±0.102*	0.133±0.092
X II	0.258±0.143	0.150±0.077	0.167±0.097*	0.130±0.094
X III	0.226±0.107	0.233±0.132**	0.120±0.059	0.125±0.048
Y I	0.273±0.156	0.215±0.095**	0.166±0.151	0.116±0.068
Y II	0.181±0.088	0.181±0.089*	0.151±0.104	0.101±0.042
Y III	0.193±0.09	0.217±0.092**	0.112±0.056	0.117±0.043

Compared with the control P<0.05* and P<0.01**

- 5 The results indicated that the extract X and Y can regulate the quantity of urine after 5 days of administration of X and Y.

After 7 days of administration of X and Y, the quantity of urine was measured at 30, 60, 120, 180, 240, 300, and 360 minutes.

- 10 The quantity of urine discharged at 30 minute by the mice receiving 200, 400 mg/kg of X and 250, 500mg/kg of Y decreased significantly (P<0.05) but increased at 240 minutes compared with the mice receiving normal saline (Table 5.3).

- 15 **Table 5.3. Effects of the extract X and Y on the quantity of urine in mice after 7 days of administration of X and Y**

Group	30min	60min	120min
Control	0.252±0.142	0.347±0.159	0.430±0.192
Positive	0.434±0.230*	0.606±0.214**	0.590±0.333
X I	0.301±0.152	0.314±0.149	0.342±0.186
X II	0.291±0.161	0.332±0.135	0.285±0.173*
X III	0.212±0.113	0.260±0.103	0.309±0.117*
Y I	0.254±0.175	0.283±0.137	0.313±0.178
Y II	0.261±0.189	0.292±0.129	0.300±0.128*
Y III	0.246±0.170	0.268±0.240	0.281±0.146*

Compared with the control P<0.05* and P<0.01**

- 20 **Table 5.3. Effects of the extract X and Y on the quantity of urine in mice after 7 days of administration of X and Y**
(continued)

Group	180min	240min	300min	360min
Control	0.285±0.136	0.155±0.119	0.122±0.071	0.111±0.061
Positive	0.314±0.119	0.279±0.192*	0.163±0.087	0.148±0.071
X I	0.267±0.179	0.200±0.114	0.176±0.147	0.157±0.077
X II	0.250±0.116	0.203±0.134	0.180±0.079*	0.129±0.085
X III	0.293±0.142	0.250±0.116*	0.194±0.104*	0.151±0.076
Y I	0.310±0.168	0.248±0.178	0.155±0.108	0.113±0.05
Y II	0.334±0.208	0.259±0.205	0.205±0.109*	0.188±0.113*
Y III	0.267±0.133	0.212±0.125	0.205±0.119*	0.169±0.073*

Compared with the control P<0.05* and P<0.01**

The results indicated that the extract X and Y can regulate the quantity of urine after 7 days of administration of X and Y.

5

After 10 days of administration of X and Y, the quantity of urine discharged at 120 minutes by the mice receiving 200, 400 mg/kg of X and 250, 500mg/kg of Y decreased significantly (P<0.05) compared with the mice receiving normal saline (Figure 3).

10

Table 5.4. Effects of the extract X and Y on the quantity of urine in mice after 10 days of administration of X and Y

Group	30min	60min	120min
Control	0.292±0.184	0.323±0.158	0.418±0.221
Positive	0.374±0.159	0.432±0.163*	0.643±0.181**
X I	0.306±0.124	0.317±0.088	0.339±0.145
X II	0.292±0.082	0.343±0.120	0.279±0.118*
X III	0.266±0.116	0.348±0.161	0.274±0.111*
Y I	0.273±0.117	0.331±0.103	0.406±0.175
Y II	0.289±0.126	0.344±0.147	0.254±0.102*
Y III	0.227±0.129	0.322±0.162	0.255±0.124*

Compared with the control P<0.05* and P<0.01**

15

Table 5.4. Effects of the extract X and Y on the quantity of urine in mice after 10 days of administration of X and T (continued)

Group	180min	240min	300min	360min
-------	--------	--------	--------	--------

Control	0.203±0.087	0.144±0.098	0.108±0.074	0.091±0.060
Positive	0.253±0.116	0.147±0.067	0.095±0.094	0.068±0.049
X I	0.249±0.094	0.172±0.093	0.120±0.058	0.093±0.050
X II	0.225±0.074	0.163±0.051	0.116±0.052	0.093±0.051
X III	0.247±0.104	0.186±0.102	0.121±0.053	0.098±0.065
Y I	0.243±0.101	0.171±0.098	0.126±0.086	0.098±0.058
Y II	0.229±0.097	0.164±0.091	0.124±0.094	0.111±0.067
Y III	0.213±0.102	0.170±0.081	0.121±0.059	0.095±0.045

Compared with the control $P < 0.05^*$ and $P < 0.01^{**}$

The results indicated that the extract X and Y can regulate the quantity of urine after 10 days of administration of X and Y.

Conclusion

The results indicated that the extract X and Y can regulate the quantity of urine after 3-10 days of administration of X and Y.

Although the present invention has been described in detail with particular reference to preferred embodiments thereof, it should be understood that the invention is capable of other different embodiments, and its details are capable of modifications in various obvious aspects. As is readily apparent to those skilled in the art, variations and modifications can be affected while remaining within the spirit and scope of the invention. Accordingly, the foregoing disclosure, description, and figures are for illustrative purpose only, and do not in any way limit the invention which is defined only by the claims.

Experiment 6:

Herb Extraction

(a) extracting Xanthoceras Sorbifolia powder of husk or branch or stem or leave or kernel or root or bark with organic solvent at ratio of 1:2 for 4-5 times for 20-35 hours for each time to form an organic extract; (b) collecting the organic extract; (c) refluxing the organic extract for 2-3 times at 80°C to form

second extracts; (d) removing the organic solvent from the second extract; and (e) Drying and sterilizing the extract to form a Xanthoceras Sorbifolia extract powder.

5 Experiment 7:

Analysis of Xanthoceras Sorbifolia extract components by HPLC chromatography

Methods and Materials

10 HPLC

A C-18 reverse phase μ bondapak column (Water P/N 27324) was equilibrated with 10% acetonitrile, 0.005% Trifluoroacetic acid (equilibration solution). An extract of Xanthoceras Sorbifolia was dissolved in equilibration solution (1 mg/ml) before being applied onto the column. 20 ug of samples was applied into column. Elution conditions: Fractions were eluted (flow rate 0.5 ml/min.) with acetonitrile (concentration gradient from 10% to 80% in 70 min) and then remains at 80% for 10 min (70-80 min). The acetonitrile then dropped to 10% (80-85 min) and remained at 10% for 25 min (85-110 min). The fractions were monitored at 207 nm with a chart speed 0.25 cm/min and OD full scale of 0.128.

Instruments: Waters Model 510 Solvent Delivery System; Waters 484 tunable Absorbance Detector; Waters 745/745B Data Module

25

Absorbance analysis

The absorption profile of Xanthoceras Sorbifolia extract at various wavelength was determined. An extract of Xanthoceras Sorbifolia was dissolved in 10% acetonitrile/TFA and scanned at 200-700 nm with a spectrophotometer [Spectronic Ins. Model Gene Sys2].

30

Results

HPLC

About 60-70 peaks can be accounted for in the profile. Among them four are major peaks, 10 are medium size and the rest are small fractions. The major peaks are labeled with a to z following increased concentration of acetonitrile elution. See Figure 1.

Absorption maximum

Three absorption maximum were identified for Xanthoceras Sorbifolia plant extract; 207nm, 278nm and 500nm. See Figure 2.

Experiment 8:

Screening of cytotoxicity of Xanthoceras Sorbifolia extract toward cancer cells derived from different human organs by MTT assay

Methods and Materials

Cells

Human cancer cell lines were obtained from American Type Culture Collection: HTB-9 (bladder), HeLa-S3 (cervix), DU145 (prostate), H460 (lung), MCF-7 (breast), K562 (leukocytes), HCT116 (colon), HepG2 (liver), U2OS (bone), T98G (brain) and OVCAR-3 (ovary). Cells were grown in culture medium (HeLa-S3, DU145, MCF-7, Hep-G2 and T98G in MEN (Earle's salts); HTB-9, H460, K562, OVCAR-3 in RPMI-1640; HCT-116, U2OS in McCoy-5A) supplemented with 10% fetal calf serum, glutamine and antibiotics in a 5% CO₂ humidified incubator at 37°C.

MTT assay

The procedure for MTT assay followed the method described in (Carmichael et al., 1987) with only minor modifications. Cells were seeded into a 96-wells plate at concentrations of 10,000/well (HTB-9, HeLa, H460, HCT116, T98G, OVCAR-3), 15,000/well (DU145, MCF-7, HepG2, U2OS), or 40,000/well (K562), for 24 hours before drug-treatment. Cells were then exposed

to drugs for 48 hours (72 hours for HepG2, U2OS, and 96 hours for MCF-7). After the drug-treatment, MTT (0.5 mg/ml) was added to cultures for an hour. The formation of formazan (product of the reduction of tetrazolium by viable cells) was dissolved with DMSO and the O.D. at 490nm was measured by an ELISA reader [Dynatech. Model MR700]. The MTT level of cells before drug-treatment was also measured (T₀). The % cell-growth (%G) is calculated as:

$$\%G = (TD - T_0 / TC - T_0) \times 100 \quad (1)$$

where TC or TD represent O.D. readings of control or drug-treated cells.

When T₀ > TD, then the cytotoxicity (LC) expressed as % of the control is calculated as:

$$\%LC = (TD - T_0 / T_0) \times 100.$$

Results

Among the 10 cell lines studies, their sensitivity toward Xanthoceras Sorbifolia extract can be divided into four groups (most sensitive: Ovary. Sensitive: bladder, bone, prostate, and leukocyte, marginal sensitive: liver, breast, and brain; and least sensitive: colon, cervix, and lung) (Figure 23) (Power point file 1-22, Slide A, B, C and D). Their IC₅₀ values are listed (Table 8.1).

Table 8.1. IC₅₀ value of Xanthoceras Sorbifolia extract determined in different cancer cells

Cancer cells from different organs	IC ₅₀ determined by MTT assay (ug/ml)
Ovary (most sensitive)	15-15
Bladder (sensitive)	45-50
Bone	40-55
Prostate	40-50
Leukocyte	45-50
Liver (marginal sensitive)	45-65

Breast	65
Brain	70-85
Colon (least sensitive)	90
Cervix	115
Lung	110

Among these cell line studied, it was found that low concentrations of the Xanthoceras Sorbifolia plant extract stimulate cell growth of bladder, bone and lung cells (Figures 3A and 3D) (PP files 1-22, slide 1 and 4).

The pure fraction Y lost the growth stimulating activity but maintained the inhibition activity (Figure 23A compare with Figure 25 and Figure 27) (PP file 4-26, slide 2 and 4). Preliminary results (Figure 25) (PP file 4-26, slide 2) indicate that the growth components resided in FPLC fractions 610 and 1116.

Experiment 9:

Purification of active Xanthoceras Sorbifolia extract

Fractionation of Xanthoceras Sorbifolia extracts components with FPLC

Methods

Column: Octadecyl functionalized silica gel; column dimension: 2cm x 28cm; equilibrated with 10% acetonitrile - 0.005% TFA. Sample loading: 1-2 ml, concentration: 100mg/ml in 10% acetonitrile/TFA.

Gradient elution: 10-80% acetonitrile in a total volume of 500 ml.

Monitor absorption wavelength: at 254nm.

Fraction Collector: 5 ml/fractions (collect from 10% to 72% acetonitrile, total 90 fractions)

Instrument: AKTA-FPLC, P920 pump; Monitor UPC-900; Frac-900.

Results

The elution profile shows 4-5 broad fractions (Figure 4) (PP-FPLC-15). These fractions were analyzed with HPLC. 5. Specific components (a-z) are then keyed back in the FPLC profile and fractions.

FPLC fractions are grouped into 7 pools and analyzed for cell growth activity in bladder cells with MTT assay. It was found 10 only one pool (#5962) contains inhibition activity (figure 5) (PP files 4-26, slide 2).

Fractionation of #5962 with FPLC - C18 open column with 64% acetonitrile isocratic elution

15

Methods

Column: Octadecyl-functionalized silica gel; 50 ml; 2cm x 28cm; equilibrated with 64% acetonitrile - 0.005% TFA.

Sample loading: 0.2 ml, with concentration: 1-2 mg/ml in 65% acetonitrile/TFA. 20

Elution: 64% acetonitrile isocratic.

Monitor absorption wavelength: at 254nm.

Fraction Collector: 1 ml fraction (collect the first 90 fractions)

25 Instrument: AKTA-FPLC, P920 pump; Monitor UPC-900; Frac-900.

Results

Fraction No. 5962 was further purified with an open ODS-C18 column with isocratic 64% acetonitrile elution. Two major 30 fractions (X and Y) were collected (Figure 26) (PP-iso80-5). MTT assay showed that only the Y fraction has the inhibition activity (Figure 27) (PP files 4-26, slide 4).

Analysis of Fraction Y with HPLC**Methods**

Column: Waters μ bondapak C18 (3.9 x 300 nm).

5 Elution: 35% or 45% isocratic elution.

Flow rate: 0.5 ml/min; 207 nm; attenuation 0.128; chart speed:
0.25 cm/min.

Results

10 On 35% isocratic analysis, the Y fraction contains 4-5 components (Y0, Y1, Y2, Y3, and Y4). Y3 and Y4 being the major components. Y1 and Y2 group together and Y3 and Y4 group together (Figure 28) (PP-HPLC-Y-iso35).

15 On 45% isocratic analysis, Y1, Y2 peaks merge into one peak, and Y3, Y4 merge into one peak (Figure 29) (PP file 6-8 slide 1). There may be a Y5 component after the Y3/4.

Final isolation of active Y component with preparative HPLC

20

Methods

Column: A preparative HPLC column (Waters Delta Pak C18-300A);

Elution: 45% acetonitrile isocratic with flow rate of 1 ml/min.

Monitor at 207nm;

25 Peak fractions were collected and lyophilized.

Results Under these conditions:

Y1 and Y2 are well separated from each other and they are collected individually. Y3/4 and Y5 are not separated (Figure
30 30) (PP-HPLC-Y-iso45). Further analysis of the ascending and the descending portion of Y3/4 peak materials with NMR analysis showed a same profile which indicated that Y3/4 is a homogenous component (Figure 31) (PP-3-4 proton NMR). Therefore the fraction is designated as Y fraction. Rerun of the collected

Y-fraction showed a single peak in HPLC with a C18 reverse phase column (Figure 32) (PP-HPLC-pure-Y).

5 Appearance and solubility. The pure Y is amorphous white powder, soluble in aqueous alcohol (methanol, ethanol), 50% acetonitrile and 100% pyridine.

MTT analysis of Y fraction indicated that:

10 (a) it has activity against ovarian cancer cells (OCAR-3) with IC50 of 1 ug/ml) which is 10-15 times more potent than the unpurified material (Figure 33a and 33b) (PP file 7-26); and

15 (b) it maintains its selectivity against ovarian cancer cells versus cervical cancer cells (HeLa) (Figure 34) (PP 5-11; compare with HeLa cells not so active).

Experiment 10

20 Determination of the chemical structure of active Xanthoceras Sorbifolia extract

Methods

NMR analysis

25 The pure Y fraction of Xanthoceras Sorbifolia extract and other samples are subjected NMR analysis. Samples were dissolved in pyridine-D5 with 0.05% v/v TMS. All NMR spectra were acquired using a Bruker Avance 600 MHz NMR spectrometer with a QXI probe ($^1\text{H}/^{13}\text{C}/^{15}\text{N}/^{31}\text{P}$) at 298 K. The numbers of scans for 1D ^1H spectra
30 were 16 to 128, depending on the sample concentration. 2D HMQC spectra were recorded with spectral widths of 6000 x 24,000 Hz and data points of 2024 x 256 for t_2 and t_1 dimensions, respectively. The numbers of scans were 4 to 128. 2D HMBC were

acquired with spectral widths of $6000 \times 30,000$ Hz and data points of 2024×512 for t_2 and t_1 dimensions, respectively. The numbers of scans were 64. The 2D data were zero-filled in t_1 dimension to double the data points, multiplied by cosine-square-bell window functions in both t_1 and t_2 dimensions, and Fourier-transformed using software XWIN-NMR. The final real matrix sizes of these 2D spectra are 2048×256 and 2048×512 data points ($F_2 \times F_1$) for HMQC and HMBC, respectively.

10 **Mass spectral analysis**

The mass of samples was analyzed by (A) MALDI-TOF Mass Spectrometry and by (B) ESI-MS Mass spectrometry.

(A) Samples for MALDI-TOF were first dissolved in acetonitrile, then mixed with the matrix CHCA (Alpha-cyano-4-hydroxycinnamic acid, 10mg CHCA/mL in 50:50 water/acetonitrile and 0.1% TFA in final concentration). The sample was dissolved completely in acetonitrile and stay dissolved after mixing with the matrix. The molecular weight was determined by the high resolution mass spectroscopy analysis with standards.

(B) For ESI, the sample was analyzed with LCQ DECA XP Plus machine made by Thermo Finnigan. It is ionized with ESI source and the solvent for the compound is acetonitrile.

25 **Results**

The profile of the proton NMR is presented in Figure 35. (Proton-NMR-7-20) The chemical shift of the proton NMR of Y is listed in Table 10.1 below.

30 **Table 10.1. Chemical Shift of the Proton NMR of Y Fraction of Xanthoceras Sorbifolia Extract**

	#	ADDRESS	FREQUENCY		INTENSITY
			[Hz]	[PPM]	
5	1	5031.9	5309.524	8.8468	0.46
	2	5430.0	5222.264	8.7015	134.66
	3	5840.9	5132.173	8.5513	0.48
	4	8204.9	4613.901	7.6878	0.26
	5	8568.1	4534.263	7.5551	88.28
10	6	8943.7	4451.919	7.4179	0.33
	7	9209.7	4393.602	7.3207	0.80
	8	9575.2	4313.488	7.1872	180.00
	9	9952.1	4230.846	7.0495	0.87
	10	10277.2	4159.585	6.9308	1.02
15	11	10886.8	4025.922	6.7081	5.68
	12	10933.6	4015.677	6.6910	6.19
	13	11939.8	3795.084	6.3235	6.26
	14	11986.7	3784.803	6.3063	5.85
	15	12576.0	3655.587	6.0910	1.47
20	16	12728.0	3622.280	6.0355	6.92
	17	12880.5	3588.844	5.9798	1.87
	18	12914.2	3581.450	5.9675	4.48
	19	12946.5	3574.366	5.9557	4.59
	20	12979.5	3567.130	5.9436	2.00
25	21	13382.6	3478.754	5.7964	2.41
	22	13415.6	3471.539	5.7844	5.03
	23	13447.3	3464.568	5.7727	5.15
	24	13479.1	3457.598	5.7611	2.66
	25	14218.8	3295.432	5.4909	14.00
30	26	14655.9	3199.603	5.3312	28.97
	27	14691.2	3191.875	5.3184	27.63
	28	15715.3	2967.359	4.9443	5.64
	29	15826.3	2943.028	4.9037	4.82
	30	15860.6	2935.504	4.8912	5.15
35	31	16047.0	2894.632	4.8231	4.20
	32	16149.6	2872.131	4.7856	3.79
	33	16171.9	2867.242	4.7775	4.42
	34	16774.0	2735.249	4.5575	5.92
	35	16856.4	2717.187	4.5274	4.26
	36	16883.5	2711.235	4.5175	4.56
	37	16903.5	2706.854	4.5102	5.89

	38	16930.6	2700.914	4.5003	4.68
	39	17021.2	2681.046	4.4672	2.99
	40	17056.9	2673.228	4.4542	8.41
	41	17099.1	2663.974	4.4388	8.87
5	42	17135.6	2655.976	4.4254	8.11
	43	17200.4	2641.771	4.4018	1.88
	44	17232.6	2634.706	4.3900	2.90
	45	17271.0	2626.288	4.3760	4.33
	46	17308.8	2618.001	4.3622	2.85
10	47	17401.8	2597.607	4.3282	0.83
	48	17459.1	2585.043	4.3073	3.41
	49	17512.6	2573.328	4.2877	4.04
	50	17596.4	2554.947	4.2571	0.89
	51	17628.2	2547.968	4.2455	0.80
15	52	17666.8	2539.518	4.2314	1.30
	53	17723.0	2527.184	4.2109	6.71
	54	17745.9	2522.172	4.2025	7.98
	55	17791.2	2512.248	4.1860	2.28
	56	17874.1	2494.071	4.1557	2.90
20	57	17895.0	2489.477	4.1480	3.09
	58	17927.1	2482.454	4.1363	2.64
	59	17948.8	2477.683	4.1284	2.51
	60	18034.1	2458.993	4.0972	3.27
	61	18064.6	2452.313	4.0861	2.96
25	62	18076.5	2449.702	4.0817	2.96
	63	18118.3	2440.538	4.0665	0.72
	64	18156.4	2432.180	4.0526	0.89
	65	18196.3	2423.432	4.0380	0.44
	66	18432.1	2371.735	3.9518	2.34
30	67	18459.7	2365.684	3.9418	4.30
	68	18487.4	2359.600	3.9316	2.24
	69	18962.4	2255.466	3.7581	3.86
	70	19011.0	2244.816	3.7404	4.38
	71	19669.3	2100.485	3.4999	4.39
35	72	19717.6	2089.898	3.4822	4.16
	73	20365.2	1947.920	3.2457	2.42
	74	20401.1	1940.054	3.2326	2.35
	75	20418.9	1936.161	3.2261	2.38
	76	20751.3	1863.290	3.1047	0.70

	77	20815.8	1849.152	3.0811	3.20
	78	20857.9	1839.919	3.0657	11.24
	79	20924.8	1825.236	3.0412	1.13
	80	23431.3	1275.730	2.1257	3.95
5	81	23491.3	1262.573	2.1037	5.36
	82	23538.7	1252.187	2.0864	15.56
	83	23570.5	1245.203	2.0748	15.89
	84	23696.1	1217.673	2.0289	2.98
	85	23770.1	1201.463	2.0019	27.17
10	86	23827.6	1188.849	1.9809	1.36
	87	23887.4	1175.734	1.9590	15.22
	88	23919.5	1168.700	1.9473	15.28
	89	24010.2	1148.811	1.9142	0.49
	90	24104.2	1128.207	1.8798	1.77
15	91	24197.7	1107.716	1.8457	28.22
	92	24263.8	1093.228	1.8216	3.27
	93	24321.8	1080.503	1.8004	2.93
	94	24373.5	1069.166	1.7815	2.83
	95	24497.0	1042.094	1.7364	26.10
20	96	24598.4	1019.871	1.6993	3.17
	97	24626.1	1013.793	1.6892	3.14
	98	24676.1	1002.826	1.6709	1.84
	99	24931.2	946.900	1.5777	2.14
	100	24983.0	935.533	1.5588	3.02
25	101	25226.8	882.103	1.4698	0.25
	102	25370.4	850.608	1.4173	4.11
	103	25412.0	841.492	1.4021	4.88
	104	25499.7	822.270	1.3701	4.07
	105	25556.8	809.746	1.3492	2.62
30	106	25639.9	791.527	1.3189	31.95
	107	25717.9	774.418	1.2904	2.84
	108	25790.4	758.539	1.2639	22.85
	109	26011.7	710.018	1.1830	4.66
	110	26070.7	697.082	1.1615	24.95
35	111	26249.2	657.953	1.0963	31.39
	112	26536.4	594.981	0.9914	25.26
	113	26610.9	578.657	0.9642	0.97
	114	26914.0	512.196	0.8534	2.04
	115	27012.2	490.676	0.8176	25.88

116	27118.1	467.463	0.7789	3.69
117	27226.4	443.715	0.7393	1.07
118	28513.4	161.554	0.2692	0.89
119	28539.8	155.777	0.2596	1.54

5

2D NMR data are presented in following figures: Figure 36 (PP-2D-NMR-Y-HMQC); Figure 37 (PP-2D-NMR-Y-HMBC); and the chemical shift data are listed in Tables 10.2 (HMQC-peak-shift) and Table 10.3 (HMBC-peak-shift) below.

10

Table 10.2. Chemical Shift Data of 2D NMR chemical shift of HMQC analysis of Y

EXPNO=5, PROCNO=1, F1PLO=144.360ppm, F1PHI=10.797ppm,
 15 F2PLO=7.966ppm, F2PHI=0.417ppm, MI=1.00cm, MAXI=10000.00cm,
 PC=1.400

#	ADDRESS	FREQUENCY		INTENSITY	#	ADDRESS	FREQUENCY		INTENSITY
	Row	[Hz] F1	[PPM] F1			row	[Hz] F1	[PPM] F1	
	Col	[Hz] F2	[PPM] F2			col	[Hz] F2	[PPM] F2	
1	148	20698.986	137.1608	2	40	670	8349.676	55.3287	2.14
	384	3574.377	5.9557		41	911	480.283	0.8003	
2	152	20564.195	136.2676	2.67	42	670	8349.676	55.3287	2.18
	401	3471.337	5.784			913	465.738	0.776	
3	157	20465.209	135.6117	45.24	43	726	7038.86	46.6427	2.51
	220	4533.779	7.5543			679	1839.659	3.0653	
4	223	18893.424	125.1963	6.22	44	726	7038.86	46.6427	1.42
	431	3295.261	5.4906			819	1016.627	1.6939	
5	234	18649.311	123.5787	100	45	726	7038.86	46.6427	2.49
	258	4311.82	7.1845			848	848.511	1.4138	
6	315	16736.119	110.9011	7.49	46	764	6151.769	40.7644	6.4
	376	3620.289	6.0322			679	1841.145	3.0678	
7	353	15834.069	104.9237	3.98	47	764	6151.769	40.7644	1.04
	493	2934.55	4.8896			682	1821.592	3.0352	
8	355	15778.398	104.5548	2.99		777	5836.727	38.6768	2.15
	449	3192.387	5.3192			850	837.869	1.3961	

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9	355	15778.398	104.5548	1.18	48	777	5836.727	38.6768	2.05
	492	2936.414	4.8927			853	818.861	1.3644	
10	451	13524.788	89.6213	2.41	49	777	5836.727	38.6768	1.64
	660	1951.827	3.2522			907	500.256	0.8335	
11	451	13524.788	89.6213	2.6	50	791	5512.022	36.5251	2.38
	663	1934.401	3.2231			775	1277.34	2.1283	
12	473	12994.274	86.1059	1.93	51	791	5512.022	36.5251	1.38
	563	2520.987	4.2005			778	1258.929	2.0977	
13	479	12861.933	85.229	3.96	52	791	5512.022	36.5251	1.38
	500	2891.933	4.8186			785	1218.892	2.0309	
14	491	12583.008	83.3807	6.95	53	791	5512.022	36.5251	1.09
	487	2967.012	4.9437			788	1201.847	2.0025	
15	523	11826.204	78.3658	3.92	54	837	4417.038	29.2693	23.74
	307	4025.464	6.7073			881	655.973	1.093	
16	523	11826.204	78.3658	4.78	55	848	4174.679	27.6633	20.53
	309	4011.801	6.6846			864	757.206	1.2617	
17	523	11826.204	78.3658	1.99	56	848	4174.679	27.6633	3.17
	545	2627.492	4.378			872	709.06	1.1815	
18	529	11690.02	77.4633	4.6	57	856	3984.149	26.4008	1.66
	504	2866.164	4.7757			774	1281.339	2.135	
19	532	11624.016	77.026	2.32	58	856	3984.149	26.4008	1.59
	530	2713.96	4.5221			778	1259.751	2.099	
20	532	11624.016	77.026	2.51	59	856	3984.149	26.4008	1.02
	532	2703.563	4.5047			804	1107.062	1.8446	
21	535	11536.339	76.445	3.34	60	856	3984.149	26.4008	1.21
	590	2363.678	3.9384			807	1089.219	1.8149	
22	545	11299.475	74.8754	2.35	61	873	3578.068	23.7099	1.68
	573	2461.387	4.1012			799	1138.137	1.8964	
23	545	11299.475	74.8754	2.1	62	873	3578.068	23.7099	1.26
	576	2447.179	4.0775			814	1046.185	1.7432	
24	555	11063.554	73.3121	6.62	63	891	3142.837	20.8259	13.95
	541	2653.693	4.4216			788	1200.655	2.0006	
25	567	10795.113	71.5333	1.84	64	891	3142.837	20.8259	28.24
	537	2673.042	4.4539			804	1107.046	1.8446	
26	567	10795.113	71.5333	2.82	65	894	3086.147	20.4502	3.08
	539	2662.683	4.4366			788	1200.275	1.9999	
					66	894	3086.147	20.4502	

27	567	10795.113	71.5333			804	1106.803	1.8442	1.17
	541	2650.933	4.417	1.72					
					67	894	3086.147	20.4502	
28	579	10495.725	69.5494			815	1041.758	1.7358	27.19
	527	2734.037	4.5555	5.06					
					68	894	3086.147	20.4502	
29	594	10156.363	67.3006			858	789.804	1.316	1.16
	563	2523.651	4.205	7.18					
					69	897	3015.337	19.981	
30	622	9486.037	62.8588			858	790.292	1.3168	33.47
	608	2256.69	3.7601	2.91					
					70	906	2802.854	18.573	
31	622	9486.037	62.8588			830	953.652	1.589	1.35
	611	2241.336	3.7346	2.67					
					71	906	2802.854	18.573	
32	622	9486.037	62.8588			834	931.208	1.5516	1.89
	635	2100.199	3.4994	2.96					
					72	914	2613.995	17.3215	
33	622	9486.037	62.8588			892	592.663	0.9875	12.71
	637	2086.756	3.477	3.42					
					73	919	2490.082	16.5004	
34	627	9381.439	62.1656			875	693.445	1.1554	8.9
	552	2586.967	4.3105	3.58					
					74	919	2490.082	16.5004	
35	627	9381.439	62.1656			880	660.343	1.1003	1.76
	555	2568.334	4.2794	3.42					
					75	925	2342.84	15.5247	
36	627	9381.439	62.1656			778	1258.345	2.0967	5.45
	568	2494.546	4.1565	2.54					
					76	925	2342.84	15.5247	
37	627	9381.439	62.1656			782	1237.122	2.0613	5.31
	571	2474.559	4.1232	2.51					
					77	925	2342.84	15.5247	
38	630	9297.809	61.6115			791	1183.41	1.9718	2.33
	531	2709.734	4.515	2.61					
					78	925	2342.84	15.5247	
39	630	9297.809	61.6115			795	1159.732	1.9324	2.38
	539	2660.734	4.4334	2.66					
					79	925	2342.84	15.5247	
						907	503.26	0.8385	6.27
					80	925	2342.84	15.5247	
						912	474.699	0.791	7.15

Table 10.3. Chemical Shift Data of 2D NMR chemical shift of HMBC analysis of Y

5 EXPNO=6, PROCNO=1
 F1PLO=178.339ppm, F1PHI=10.721ppm, F2PLO=6.881ppm,
 F2PHI=0.573ppm
 MI=1.00cm, MAXI=10000.00cm, PC=1.400

#	ADDRESS	FREQUENCY		INTENSITY	#	ADDRESS	FREQUENCY		INTENSITY
	Row Col	[Hz/F1] [Hz/F2]	[PPM/F1] [PPM/F2]			Row Col	[Hz/F1] [Hz/F2]	[PPM/F1] [PPM/F2]	
1	123	26590.75	176.2058	3.11	181	814	6218.377	41.2066	1.66
	895	573.276	0.9552			880	659.072	1.0982	
2	145	25939.373	171.8894	2.92	182	814	6218.377	41.2066	49.75
	531	2710.686	4.5166			891	594.287	0.9902	
3	145	25939.373	171.8894	2.54	183	814	6218.377	41.2066	2.16
	539	2662.033	4.4355			895	572.724	0.9543	
4	166	25312.006	167.7321	3.45	184	814	6218.377	41.2066	1.62
	308	4016.437	6.6923			909	489.811	0.8161	
5	166	25312.006	167.7321	14.9	185	823	5950.421	39.431	1.07
	346	3793.847	6.3214			662	1940.805	3.2338	
6	166	25312.006	167.7321	12.47	186	823	5950.421	39.431	1.62
	348	3785.322	6.3072			776	1271.011	2.1178	
7	166	25312.006	167.7321	1.47	187	823	5950.421	39.431	2.29
	385	3564.443	5.9392			804	1107.587	1.8455	
8	166	25312.006	167.7321	4.16	188	823	5950.421	39.431	1.04
	400	3477.948	5.795			831	947.726	1.5791	
9	166	25312.006	167.7321	3.96	189	823	5950.421	39.431	1.45
	403	3458.552	5.7627			858	790.52	1.3172	
10	166	25312.006	167.7321	1.17	190	823	5950.421	39.431	54.14
	781	1243.983	2.0728			864	757.09	1.2615	
11	166	25312.006	167.7321	10.44	191	823	5950.421	39.431	72.55
	788	1200.447	2.0002			874	696.048	1.1598	
12	166	25312.006	167.7321	3.35	192	823	5950.421	39.431	9.62
	793	1171.992	1.9528			880	658.503	1.0972	
13	166	25312.006	167.7321	33.24	193	823	5950.421	39.431	2.75
	815	1041.336	1.7351			891	594.53	0.9906	
14	290	21640.068	143.3997	5.67	194	823	5950.421	39.431	7.32
	679	1839.97	3.0658			911	479.432	0.7988	
15	290	21640.068	143.3997	1.03	195	823	5950.421	39.431	7.03
	788	1199.609	1.9988			913	465.776	0.7761	
16	290	21640.068	143.3997						

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	804	1107.222	1.8449	33.9	196	827	5834.797	38.6648	
						804	1106.687	1.844	2.69
17	290	21640.068	143.3997			827	5834.797	38.6648	
	813	1053.918	1.7561	2.3	197	819	1017.803	1.6959	1.66
18	290	21640.068	143.3997			827	5834.797	38.6648	
	848	848.155	1.4132	1.25	198	822	1001.708	1.6691	1.68
19	322	20697.354	137.1527			827	5834.797	38.6648	
	780	1246.505	2.077	31.46	199	841	889.985	1.4829	1.01
20	322	20697.354	137.1527			827	5834.797	38.6648	
	788	1200.075	1.9996	44.6	200	858	789.621	1.3157	1.33
21	322	20697.354	137.1527			827	5834.797	38.6648	
	793	1170.377	1.9501	3.79	201	864	758.164	1.2633	3.94
22	322	20697.354	137.1527			827	5834.797	38.6648	
	815	1040.86	1.7343	4.31	202	874	696.137	1.1599	6.09
23	327	20566.367	136.2847			827	5834.797	38.6648	
	780	1246.629	2.0772	3.06	203	881	656.905	1.0945	2.43
24	327	20566.367	136.2847			827	5834.797	38.6648	
	788	1201.192	2.0015	5.78	204	892	593.606	0.9891	1.9
25	327	20566.367	136.2847			827	5834.797	38.6648	
	793	1170.748	1.9507	47.25	205	909	489.245	0.8152	51.13
26	327	20566.367	136.2847			830	5743.329	38.0586	
	815	1041.067	1.7346	58.19	206	804	1108.031	1.8462	1.4
27	365	19434.006	128.7811			830	5743.329	38.0586	
	780	1245.861	2.0759	16.02	207	858	790.551	1.3172	1.95
28	365	19434.006	128.7811			830	5743.329	38.0586	
	788	1200.442	2.0002	33.73	208	864	757.091	1.2615	2.85
29	365	19434.006	128.7811			830	5743.329	38.0586	
	793	1171.204	1.9515	46.7	209	874	696.052	1.1598	3.54
30	365	19434.006	128.7811			830	5743.329	38.0586	
	815	1040.94	1.7344	80.6	210	881	655.62	1.0924	2.41
31	384	18893.113	125.1968			830	5743.329	38.0586	
	679	1839.547	3.0651	9.8	211	891	594.762	0.991	3.06
32	384	18893.113	125.1968			830	5743.329	38.0586	
	788	1200.729	2.0007	2.34	212	909	489.474	0.8156	10.76
33	384	18893.113	125.1968			834	5623.896	37.2672	
	801	1124.942	1.8744	1.93	213	804	1107.433	1.8452	1.25
34	384	18893.113	125.1968			834	5623.896	37.2672	
	803	1113.96	1.8561	1.36	214				

						822	1002.039	1.6696	1.43
35	384	18893.113	125.1968						
	809	1077.556	1.7954	3.32	215	834	5623.896	37.2672	
						833	939.62	1.5656	1.01
36	384	18893.113	125.1968						
	813	1051.694	1.7524	3.14	216	834	5623.896	37.2672	
						858	790.603	1.3173	4.03
37	384	18893.113	125.1968						
	848	847.499	1.4121	1.45	217	834	5623.896	37.2672	
						864	756.768	1.2609	1.89
38	457	16738.236	110.9173						
	361	3707.331	6.1772	6.44	218	834	5623.896	37.2672	
						874	696.444	1.1604	2.15
39	457	16738.236	110.9173						
	390	3534.169	5.8887	6.75	219	834	5623.896	37.2672	
						881	656.011	1.0931	4.39
40	457	16738.236	110.9173						
	486	2972.803	4.9534	1.24	220	834	5623.896	37.2672	
						891	594.776	0.991	4.19
41	457	16738.236	110.9173						
	488	2962.223	4.9357	1.4	221	834	5623.896	37.2672	
						909	489.272	0.8152	10.53
42	457	16738.236	110.9173						
	563	2520.559	4.1998	12.21	222	837	5533.835	36.6704	
						777	1268.681	2.1139	1.72
43	488	15822.76	104.8508						
	531	2712.055	4.5189	1.39	223	837	5533.835	36.6704	
						804	1108.282	1.8466	1.01
44	488	15822.76	104.8508						
	538	2668.887	4.447	4.68	224	837	5533.835	36.6704	
						811	1067.12	1.7781	1.7
45	488	15822.76	104.8508						
	545	2627.709	4.3783	14.61	225	837	5533.835	36.6704	
						819	1017.407	1.6952	4.37
46	488	15822.76	104.8508						
	660	1952.474	3.2533	2.34	226	837	5533.835	36.6704	
						822	1001.73	1.6691	4.67
47	488	15822.76	104.8508						
	662	1941.101	3.2343	3.52	227	837	5533.835	36.6704	
						832	943.762	1.5725	2.78
48	488	15822.76	104.8508						
	664	1928.93	3.214	2.41	228	837	5533.835	36.6704	
						849	841.934	1.4028	2.09
49	489	15777.47	104.5507						
	538	2669.101	4.4473	12.69	229	837	5533.835	36.6704	
						858	790.63	1.3174	8.57
50	489	15777.47	104.5507						
	546	2622.941	4.3704	6.06	230	837	5533.835	36.6704	
						864	757.963	1.2629	2.1
51	489	15777.47	104.5507						
	590	2363.695	3.9384	3.08	231	837	5533.835	36.6704	
						874	695.245	1.1584	1.83
52	489	15777.47	104.5507						
	660	1953.443	3.2549	2.02	232	837	5533.835	36.6704	
						881	656.351	1.0936	11.03

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53	489	15777.47	104.5507	1.47	233	837	5533.835	36.6704	25.74
	662	1940.913	3.234			891	594.692	0.9909	
54	566	13527.92	89.6439	11.15	234	837	5533.835	36.6704	53.53
	492	2938.198	4.8957			909	489.139	0.815	
55	566	13527.92	89.6439	1.93	235	837	5533.835	36.6704	6.55
	805	1101.852	1.8359			913	467.893	0.7796	
56	566	13527.92	89.6439	5.09	236	840	5450.419	36.1177	5.35
	851	830.552	1.3839			307	4023.448	6.704	
57	566	13527.92	89.6439	53.64	237	840	5450.419	36.1177	5.05
	864	757.374	1.262			309	4013.866	6.688	
58	566	13527.92	89.6439	39.08	238	840	5450.419	36.1177	1.25
	874	696.11	1.1599			346	3794.546	6.3226	
59	566	13527.92	89.6439	4.62	239	840	5450.419	36.1177	3.88
	880	658.836	1.0978			679	1839.242	3.0646	
60	566	13527.92	89.6439	1.38	240	840	5450.419	36.1177	6.34
	911	480.432	0.8005			849	842.83	1.4043	
61	566	13527.92	89.6439	1.4	241	840	5450.419	36.1177	88.69
	913	466.506	0.7773			858	790.237	1.3167	
62	584	12992.842	86.0981	5.29	242	840	5450.419	36.1177	1.79
	376	3621.487	6.0342			862	767.125	1.2782	
63	584	12992.842	86.0981	1.17	243	840	5450.419	36.1177	1.99
	531	2708.564	4.5131			864	755.041	1.2581	
64	584	12992.842	86.0981	4.18	244	840	5450.419	36.1177	2.24
	539	2662.648	4.4366			874	695.441	1.1588	
65	584	12992.842	86.0981	6.92	245	840	5450.419	36.1177	84.89
	545	2625.947	4.3754			881	656.06	1.0931	
66	584	12992.842	86.0981	1.36	246	840	5450.419	36.1177	7.64
	864	757.04	1.2614			891	594.675	0.9909	
67	588	12860.941	85.2241	2.76	247	840	5450.419	36.1177	8.88
	370	3655.044	6.0901			909	489.3	0.8153	
68	588	12860.941	85.2241	10.88	248	875	4418.238	29.2778	8.21
	376	3621.609	6.0344			307	4022.778	6.7028	
51	489	15777.47	104.5507	1.21	249	875	4418.238	29.2778	7.83
69	588	12860.941	85.2241			309	4014.385	6.6889	
70	588	12860.941	85.2241	1.05	250	875	4418.238	29.2778	1.85
	864	756.227	1.26			679	1841.576	3.0685	

71	598	12585.614	83.3996	1.92	251	875	4418.238	29.2778	1.65
	376	3621.268	6.0338			849	844.669	1.4074	
72	598	12585.614	83.3996	1.23	252	875	4418.238	29.2778	100
	475	3038.901	5.0635			858	790.492	1.3171	
73	598	12585.614	83.3996	1.6	253	875	4418.238	29.2778	3.72
	500	2890.984	4.817			863	763.811	1.2727	
74	598	12585.614	83.3996	3.39	254	875	4418.238	29.2778	22.51
	505	2863.233	4.7708			870	718.885	1.1978	
75	598	12585.614	83.3996	1.11	255	875	4418.238	29.2778	4.9
	881	655.156	1.0916			875	691.903	1.1529	
76	623	11822.784	78.3447	2.3	256	875	4418.238	29.2778	1.92
	295	4093.818	6.8212			878	670.147	1.1166	
77	623	11822.784	78.3447	2.25	257	875	4418.238	29.2778	1.77
	321	3940.803	6.5663			882	648.47	1.0805	
78	623	11822.784	78.3447	18.28	258	875	4418.238	29.2778	19.66
	348	3785.12	6.3069			892	593.301	0.9886	
79	623	11822.784	78.3447	1.13	259	883	4176.082	27.6732	1.71
	434	3277.04	5.4603			662	1941.3	3.2346	
80	623	11822.784	78.3447	7.26	260	883	4176.082	27.6732	15.64
	448	3194.918	5.3234			853	819.336	1.3652	
81	623	11822.784	78.3447	7.36	261	883	4176.082	27.6732	4.59
	563	2521.171	4.2008			857	794.801	1.3243	
82	623	11822.784	78.3447	1.26	262	883	4176.082	27.6732	1.7
	845	867.271	1.4451			863	763.374	1.272	
83	623	11822.784	78.3447	10.09	263	883	4176.082	27.6732	89.76
	849	844.669	1.4074			874	696.058	1.1598	
84	623	11822.784	78.3447	26.63	264	883	4176.082	27.6732	2.96
	858	790.448	1.3171			878	671.861	1.1195	
85	623	11822.784	78.3447	51.16	265	883	4176.082	27.6732	7.96
	881	656.434	1.0938			881	657.198	1.095	
86	630	11619.674	76.9987	1.21	266	883	4176.082	27.6732	2.54
	348	3785.085	6.3068			911	479.487	0.7989	
87	630	11619.674	76.9987	6.49	267	883	4176.082	27.6732	2.27
	539	2661.936	4.4354			913	466.645	0.7775	
88	630	11619.674	76.9987	1.49	268	903	3588.775	23.7813	2.85
	552	2584.607	4.3065			431	3296.117	5.4921	
89	630	11619.674	76.9987		269	903	3588.775	23.7813	

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	858	790.978	1.3179	2.74		791	1182.756	1.9707	1.05
90	630	11619.674	76.9987		270	903	3588.775	23.7813	
	881	655.977	1.093	5.21		801	1127.886	1.8793	1.45
91	641	11304.499	74.9102		271	903	3588.775	23.7813	
	307	4021.328	6.7004	1.03		804	1108.54	1.8471	1.43
92	641	11304.499	74.9102		272	903	3588.775	23.7813	
	526	2736.013	4.5588	4.14		811	1064.128	1.7731	1.37
93	641	11304.499	74.9102		273	903	3588.775	23.7813	
	538	2670.608	4.4498	9.8		821	1006.1	1.6764	3.82
94	641	11304.499	74.9102		274	903	3588.775	23.7813	
	881	656.537	1.0939	1.61		874	696.649	1.1608	2.42
95	650	11028.925	73.0841		275	903	3588.775	23.7813	
	308	4015.948	6.6915	16.79		881	655.757	1.0926	2.43
96	650	11028.925	73.0841		276	917	3174.632	21.037	
	346	3793.459	6.3207	13.94		307	4021.419	6.7006	1.44
97	650	11028.925	73.0841		277	917	3174.632	21.037	
	526	2736.761	4.5601	7.46		384	3571.435	5.9508	2.36
98	650	11028.925	73.0841		278	917	3174.632	21.037	
	574	2454.41	4.0896	4.7		403	3463.331	5.7707	1.11
99	650	11028.925	73.0841		279	917	3174.632	21.037	
	610	2245.856	3.7421	11.08		561	2531.85	4.2186	5.21
100	650	11028.925	73.0841		280	917	3174.632	21.037	
	637	2088.895	3.4806	5.03		564	2517.7	4.195	5.22
101	650	11028.925	73.0841		281	917	3174.632	21.037	
	679	1839.51	3.065	8.83		778	1262.748	2.104	3.52
102	650	11028.925	73.0841		282	917	3174.632	21.037	
	848	850.226	1.4167	1.52		793	1170.055	1.9496	22.51
103	650	11028.925	73.0841		283	917	3174.632	21.037	
	881	656.986	1.0947	1.8		800	1133.275	1.8883	2.77
104	658	10790.329	71.503		284	917	3174.632	21.037	
	308	4016.477	6.6923	1.26		805	1100.419	1.8335	3.08
105	658	10790.329	71.503		285	917	3174.632	21.037	
	531	2708.84	4.5135	2.58		809	1079.427	1.7986	2.91
106	658	10790.329	71.503		286	917	3174.632	21.037	
	564	2518.203	4.1959	5.49		815	1043.118	1.7381	24.13
107	658	10790.329	71.503		287	917	3174.632	21.037	
	609	2253.841	3.7554	1.07		827	975.02	1.6246	2.05

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108	658 881	10790.329 655.677	71.503 1.0925	1.26	288	917 848	3174.632 851.242	21.037 1.4184	1.6
109	668 530	10496.015 2715.37	69.5527 4.5244	1.73	289	917 864	3174.632 757.267	21.037 1.2618	1.67
110	668 533	10496.015 2699.76	69.5527 4.4984	1.85	290	917 869	3174.632 727.306	21.037 1.2119	1.8
111	668 540	10496.015 2658.708	69.5527 4.43	3.19	291	917 881	3174.632 656.407	21.037 1.0937	6.11
112	668 590	10496.015 2363.392	69.5527 3.9379	4.52	292	922 309	3013.427 4014.743	19.9687 6.6895	13.55
113	668 804	10496.015 1107.602	69.5527 1.8455	1	293	922 401	3013.427 3472.374	19.9687 5.7857	2.11
114	668 881	10496.015 656.508	69.5527 1.0939	1.01	294	922 679	3013.427 1841.875	19.9687 3.069	7.26
115	680 541	10155.364 2651.054	67.2954 4.4172	2.15	295	922 777	3013.427 1263.952	19.9687 2.106	1.96
116	680 804	10155.364 1107.228	67.2954 1.8449	32.23	296	922 794	3013.427 1165.725	19.9687 1.9424	1.57
117	680 881	10155.364 656.545	67.2954 1.0939	1	297	922 799	3013.427 1137.94	19.9687 1.8961	2.13
118	703 346	9486.47 3793.169	62.8629 6.3203	9.4	298	922 804	3013.427 1104.456	19.9687 1.8403	3.32
119	703 348	9486.47 3784.896	62.8629 6.3065	9.63	299	922 815	3013.427 1043.07	19.9687 1.738	1.92
120	703 679	9486.47 1839.346	62.8629 3.0648	3.08	300	922 826	3013.427 975.688	19.9687 1.6257	3.29
121	703 863	9486.47 758.265	62.8629 1.2634	1.07	301	922 847	3013.427 852.676	19.9687 1.4207	19.79
122	706 347	9376.976 3790.748	62.1373 6.3162	1.61	302	922 863	3013.427 759.553	19.9687 1.2656	3.39
123	706 505	9376.976 2862.456	62.1373 4.7695	2.79	303	922 869	3013.427 726.647	19.9687 1.2108	19.23
124	706 881	9376.976 655.399	62.1373 1.092	1.01	304	922 881	3013.427 656.158	19.9687 1.0933	72.09
125	709 590	9296.042 2363.684	61.601 3.9384	7.53	305	929 308	2803.19 4021.188	18.5756 6.7002	1.12
126	741	8346.307	55.3075		306	929	2803.19	18.5756	

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	777	1264.802	2.1074	3.77		823	997.155	1.6615	1.03
127	741	8346.307	55.3075		307	929	2803.19	18.5756	
	852	825.908	1.3761	3.3		847	852.409	1.4203	2.46
128	741	8346.307	55.3075		308	929	2803.19	18.5756	
	864	757.401	1.262	35.15		864	756.417	1.2604	2.78
129	741	8346.307	55.3075		309	929	2803.19	18.5756	
	874	696.341	1.1603	25.71		869	728.727	1.2142	2.15
130	741	8346.307	55.3075		310	929	2803.19	18.5756	
	881	658.199	1.0967	3.92		881	657.345	1.0953	5.08
131	741	8346.307	55.3075		311	929	2803.19	18.5756	
	891	595.647	0.9925	1.15		902	531.583	0.8857	1.25
132	741	8346.307	55.3075		312	929	2803.19	18.5756	
	909	489.518	0.8156	23.33		911	478.022	0.7965	4.88
133	781	7163.191	47.4675		313	929	2803.19	18.5756	
	346	3793.181	6.3203	1.69		913	466.494	0.7773	5.42
134	781	7163.191	47.4675		314	936	2611.793	17.3073	
	431	3294.477	5.4893	5.3		777	1266.005	2.1094	1.92
135	781	7163.191	47.4675		315	936	2611.793	17.3073	
	539	2661.476	4.4346	3.1		783	1228.491	2.0469	2.67
136	781	7163.191	47.4675		316	936	2611.793	17.3073	
	541	2647.966	4.4121	3.42		786	1211.785	2.0191	2.98
137	781	7163.191	47.4675		317	936	2611.793	17.3073	
	564	2517.984	4.1955	1.14		819	1018.301	1.6967	5.9
138	781	7163.191	47.4675		318	936	2611.793	17.3073	
	679	1839.442	3.0649	3.64		822	1001.86	1.6693	7.01
139	781	7163.191	47.4675		319	936	2611.793	17.3073	
	804	1107.164	1.8448	37.94		864	757.418	1.262	6.44
140	781	7163.191	47.4675		320	936	2611.793	17.3073	
	822	1002.093	1.6697	1.62		869	725.753	1.2093	1.48
141	781	7163.191	47.4675		321	936	2611.793	17.3073	
	849	845.058	1.4081	2.98		872	707.264	1.1785	1.56
142	781	7163.191	47.4675		322	936	2611.793	17.3073	
	858	790.203	1.3167	4.12		881	654.782	1.091	17.83
143	781	7163.191	47.4675		323	936	2611.793	17.3073	
	874	695.441	1.1588	1.07		902	531.793	0.8861	19.86
144	781	7163.191	47.4675		324	936	2611.793	17.3073	
	881	656.256	1.0935	6.01					

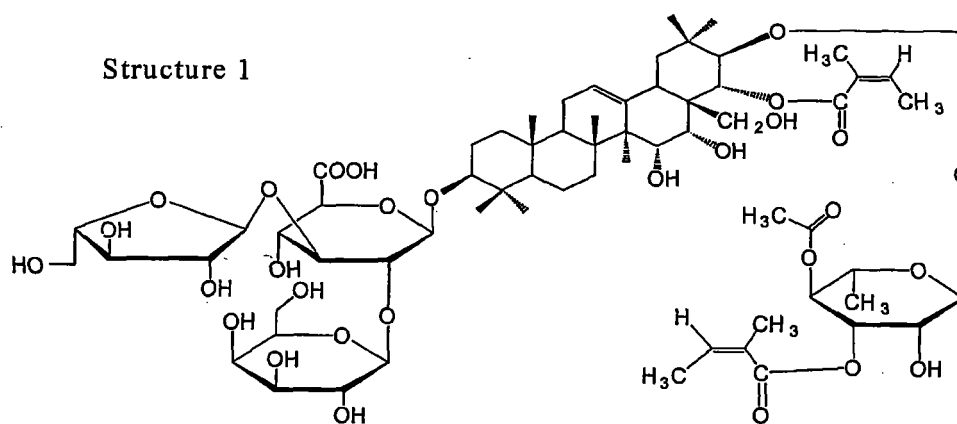
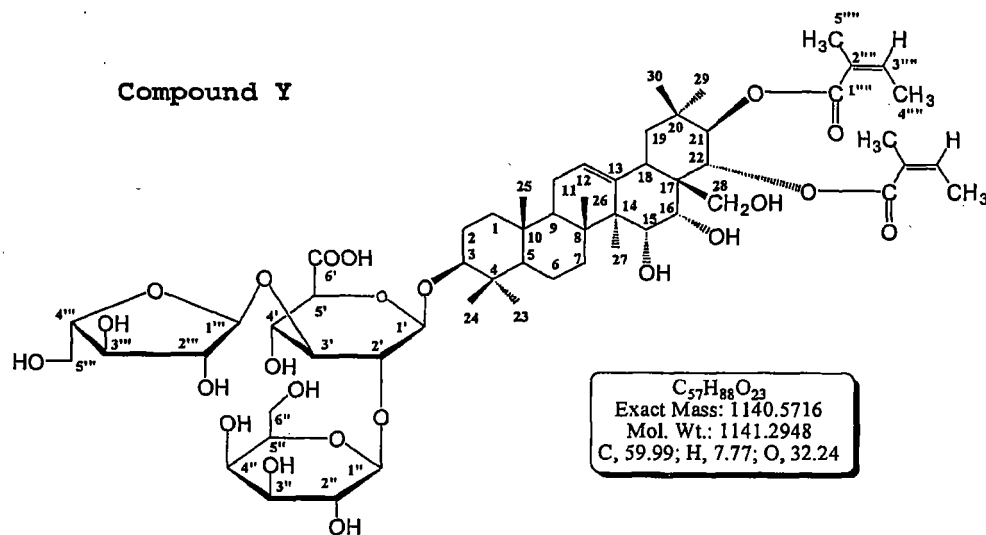
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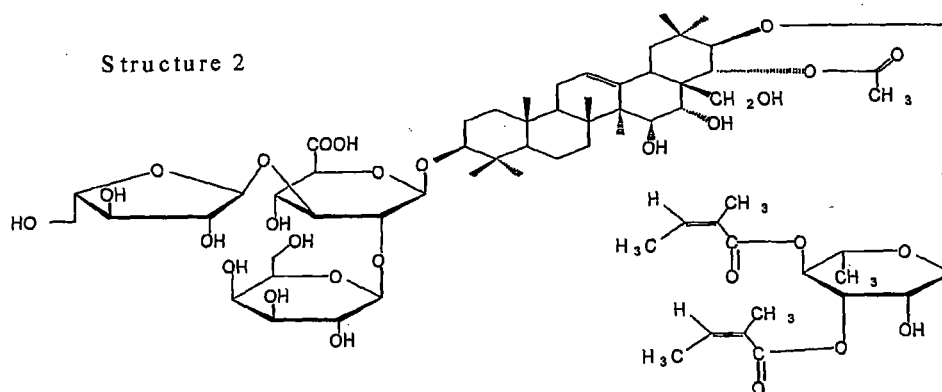
145	781	7163.191	47.4675	39.6	325	909	490.194	0.8168	2.94
	891	594.695	0.9909			936	2611.793	17.3073	
146	781	7163.191	47.4675	3.9	326	920	427.649	0.7126	1.52
	909	489.613	0.8158			940	2489.586	16.4974	
147	786	7041.051	46.6581	2.39	327	662	1941.819	3.2355	2.46
	432	3290.762	5.4831			940	2489.586	16.4974	
148	786	7041.051	46.6581	1.21	328	664	1929.172	3.2144	1.76
	669	1898.191	3.1628			940	2489.586	16.4974	
149	786	7041.051	46.6581	2.37	329	822	1001.01	1.6679	1.2
	677	1850.653	3.0836			940	2489.586	16.4974	
150	786	7041.051	46.6581	2.84	330	864	757.021	1.2614	66
	680	1835.948	3.0591			940	2489.586	16.4974	
151	786	7041.051	46.6581	2.66	331	871	712.622	1.1874	10.06
	682	1821.518	3.0351			940	2489.586	16.4974	
152	786	7041.051	46.6581	2.02	332	880	660.618	1.1007	2.75
	689	1782.432	2.9699			940	2489.586	16.4974	
153	786	7041.051	46.6581	2.05	333	885	632.387	1.0537	18.76
	776	1274.214	2.1231			940	2489.586	16.4974	
154	786	7041.051	46.6581	1.51	334	891	594.244	0.9901	3.19
	800	1129.576	1.8821			940	2489.586	16.4974	
155	786	7041.051	46.6581	6.33	335	899	548.142	0.9133	1.83
	804	1106.939	1.8444			940	2489.586	16.4974	
156	786	7041.051	46.6581	3.27	336	911	478.615	0.7975	8.03
	808	1082.787	1.8042			940	2489.586	16.4974	
157	786	7041.051	46.6581	3.51	337	913	466.429	0.7772	10.1
	811	1065.178	1.7748			940	2489.586	16.4974	
158	786	7041.051	46.6581	3.1	338	920	426.462	0.7106	1.57
	829	959.508	1.5988			945	2348.374	15.5617	
159	786	7041.051	46.6581	1.42	339	383	3576.612	5.9594	2.25
	838	906.837	1.511			945	2348.374	15.5617	
160	786	7041.051	46.6581	1.15	340	402	3464.839	5.7732	1.77
	848	847.28	1.4118			945	2348.374	15.5617	
161	786	7041.051	46.6581	49.82	341	769	1311.156	2.1847	9.92
	858	790.53	1.3172			945	2348.374	15.5617	
162	786	7041.051	46.6581	1.14	342	783	1233.208	2.0548	4.9
	874	696.77	1.161			945	2348.374	15.5617	
						791	1184.039	1.9729	8.41

163	786	7041.051	46.6581	70.13	343	945	2348.374	15.5617	5.06
	881	656.443	1.0938			804	1106.429	1.8436	
164	786	7041.051	46.6581	18.99	344	945	2348.374	15.5617	3.91
	891	594.532	0.9906			819	1018.365	1.6968	
165	786	7041.051	46.6581	21.1	345	945	2348.374	15.5617	5.15
	909	489.363	0.8154			822	1001.834	1.6693	
166	814	6218.377	41.2066	1.45	346	945	2348.374	15.5617	6.6
	431	3293.885	5.4883			864	756.741	1.2609	
167	814	6218.377	41.2066	3.03	347	945	2348.374	15.5617	1.2
	561	2531.574	4.2182			872	710.417	1.1837	
168	814	6218.377	41.2066	3.26	348	945	2348.374	15.5617	2.79
	564	2517.867	4.1953			881	655.039	1.0914	
169	814	6218.377	41.2066	2.64	349	945	2348.374	15.5617	2.24
	635	2096.944	3.494			885	632.486	1.0539	
170	814	6218.377	41.2066	2.51	350	945	2348.374	15.5617	19.61
	678	1846.079	3.076			899	550.147	0.9167	
171	814	6218.377	41.2066	1.75	351	945	2348.374	15.5617	3.78
	776	1274.484	2.1236			906	510.518	0.8506	
172	814	6218.377	41.2066	1.41	352	945	2348.374	15.5617	8.74
	783	1228.144	2.0464			911	480.301	0.8003	
173	814	6218.377	41.2066	1.51	353	945	2348.374	15.5617	8.49
	786	1211.726	2.019			914	464.362	0.7737	
174	814	6218.377	41.2066	1.43	354	945	2348.374	15.5617	18.76
	799	1136.142	1.8931			920	426.538	0.7107	
175	814	6218.377	41.2066	67.13					
	804	1107.204	1.8448						
176	814	6218.377	41.2066	4.37					
	819	1018.301	1.6967						
177	814	6218.377	41.2066	4.96					
	822	1001.489	1.6687						
178	814	6218.377	41.2066	3.66					
	833	939.695	1.5657						
179	814	6218.377	41.2066	2.02					
	864	756.271	1.2601						
180	814	6218.377	41.2066	2.98					
	874	695.963	1.1596						

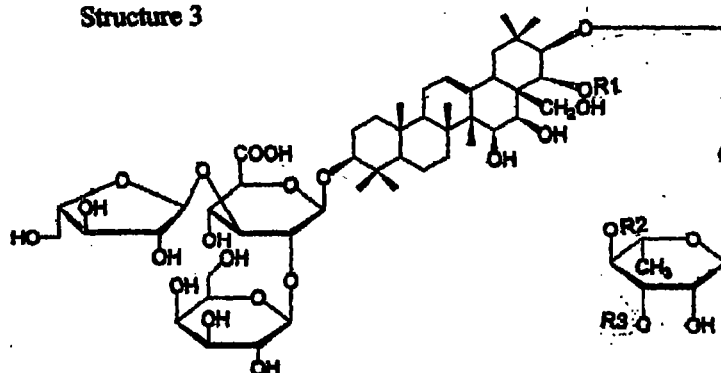
Table 10.4 (Y-structure and 2D NMR chemical shift data) shows the assignment of functional groups derived from the chemical shifts of proton and carbon-13 of compound Y. Based on these data and analysis, the structure of compound Y is assigned and shown below.



Structure 2



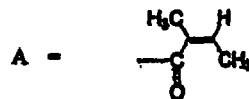
Structure 3



Where R1 = A or B or C

R2 = A or B or C

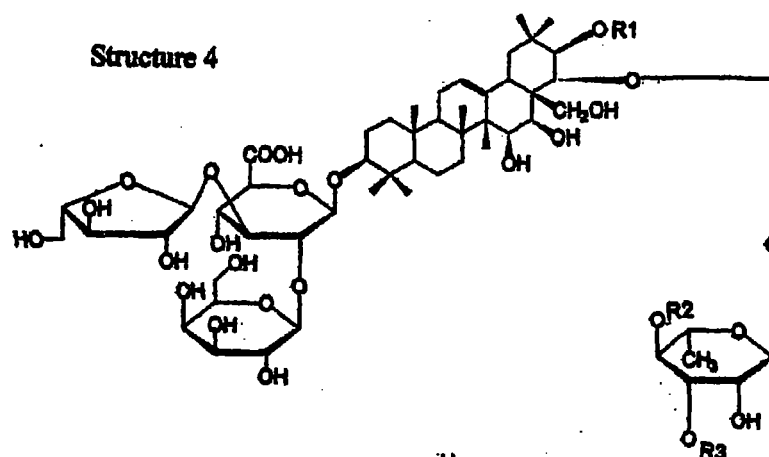
R3 = A or B or C



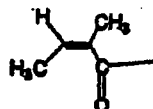
	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C



A =



Where R1 = A or B or C

R2 = A or B or C

R3 = A or B or C

B =



C = H

	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

Structure of Compound Y:

The chemical name of compound Y is:

3-O- [β-D-galactopyranosyl (1→2)] -α-L-arabinofuranosyl (1→3) -
 5 β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β,
 22α, 28-hexahydroxyolean-12-ene

Table 10.4. ^{13}C and ^1H NMR data for compound Y (in Pyridine- d_5)^a

Position	C	H	Key HMBC correlations
1	38.7	0.83, 1.40	C-3, C-5, C-9
2	26.4	1.81, 2.14	-
3	89.6	3.25, 1H, dd, 12.0/4.0 Hz	C-23, C-24, GlcA C-1'
4	39.4	-	-
5	55.3	0.78	-
6	18.5	1.55, 1.59	C-8, C-10
7	36.5	2.00, 2.10	C-5, C-9
8	41.2	-	-
9	47.0	3.06	C-7, C-8, C-12, C-14, C-26
10	37.2	-	-
11	23.7	1.74, 1.89	-
12	125.2	5.49, 1H, br s	C-9, C-11, C-14, C-18
13	143.4	-	-
14	47.5	-	-
15	67.3	4.21	C-8, C-27
16	73.6	4.45	C-14, C-15, C-18
17	48.3	-	-
18	40.8	3.07	C-12, C-13, C-14, C-16, C-19, C-20, C-28,
19	46.8	1.41, 1.69	-
20	36.2	-	-
21	79.3	6.71, 1H, d, 10 Hz	C-20, C-22, C-29, C-30, 21-O-Ang C-1''''
22	73.5	6.32, 1H, d, 10 Hz	C-16, C-17, C-21, C-28, 22-O-Ang C-1''''
23	27.7	1.26, 3H, s	C-3, C-4, C-5, C-24
24	16.5	1.16, 3H, s	C-3, C-4, C-5, C-23
25	16.0	0.81, 3H, s	C-1, C-5, C-9, C-10
26	17.3	0.99, 3H, s	C-7, C-8, C-9, C-14
27	21.0	1.85, 3H, s	C-8, C-13, C-14, C-15
28	62.9	3.50, 1H, d, 11.0 Hz, 3.76, 1H, d, 11.0 Hz,	C-16, C-17, C-18, C-22
29	29.2	1.09, 3H, s	C-19, C-20, C-21, C-30
30	20.0	1.32, 3H, s	C-19, C-20, C-21, C-29
GlcA			

1'	104.9	4.89, 1H, d, 7.8 Hz	C-3
2'	79.1	4.38	GlcA C-1', C-3', Gal C-1"
3'	86.1	4.20	GlcA C-2', C-4', Ara C-1'''
4'	71.5	4.42	GlcA C-3', C-5', C-6'
5'	78.0	4.52	GlcA C-4', C-6'
6'	171.9	-	-
Gal			
1"	104.6	5.32, 1H, d, 7.7 Hz	GlcA C-2'
2"	73.6	4.42	Gal C-1", C-3"
3"	74.9	4.10	Gal C-2"
4"	69.5	4.56	Gal C-2", C-3"
5"	76.4	3.94	Gal C-4", C-6"
6"	61.6	4.43, 4.52	Gal C-4", C-5"
Ara-f			
1'''	110.6	6.03, 1H, br s	GlcA C-3', Ara C-2''', C-4'''
2'''	83.4	4.94	Ara C-3'''
3'''	78.3	4.78	Ara C-2'''
4'''	85.2	4.82	Ara C-5'''
5'''	62.2	4.12, 4.28	Ara C-3'''
21-O-Ang			
1''''	167.7	-	-
2''''	129.6	-	-
3''''	137.2	5.96, 1H, dq, 7.0/1.5 Hz	Ang C-1''', C-4''', C-5''''
4''''	15.5	2.10, 3H, dq, 7.0/1.5 Hz	Ang C-2''', C-3''''
5''''	20.8	2.00, 3H, s	Ang C-1''', C-2''', C-3''''
22-O-Ang			
1''''	167.9	-	-
2''''	129.8	-	-
3''''	136.3	5.78, 1H, dq, 7.0/1.5 Hz	Ang C-1''', C-4''', C-5''''
4''''	15.5	1.93, 3H, dq, 7.0/1.5 Hz	Ang C-2''', C-3''''
5''''	20.5	1.74, 3H, s	Ang C-1''', C-2''', C-3''''

^a The data were assigned based on HMQC and HMBC correlations.

Figure 38 shows the mass spectrum of Y as determined by MALDI-TOF and ESI-MS techniques.

5 **Conclusion**

Based on the chemical shift analysis, the active compound Y isolated from extract of *Xanthoceras Sorbifolia* is a triterpenoid saponins with three sugars and biangeloyl groups attached to the backbone. The formula of Y is C₅₇H₈₈O₂₃, and
10 the chemical name of Y is:

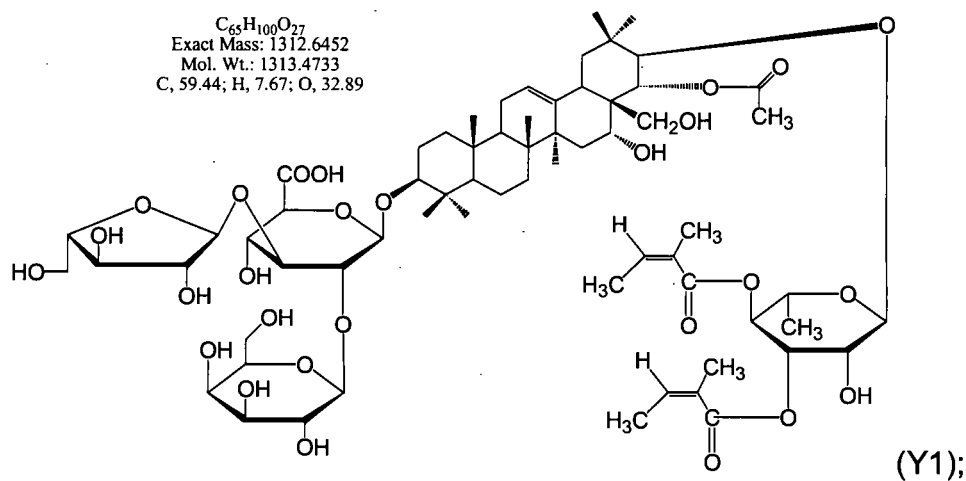
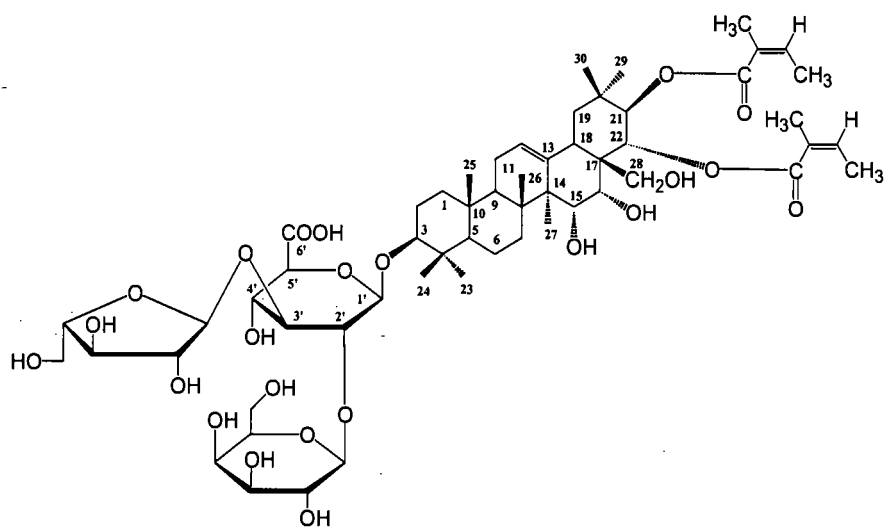
3-O- [β-D-galactopyranosyl (1→2)] -α-L-arabinofuranosyl (1→3) -
β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β,
22α, 28-hexahydroxyolean-12-ene

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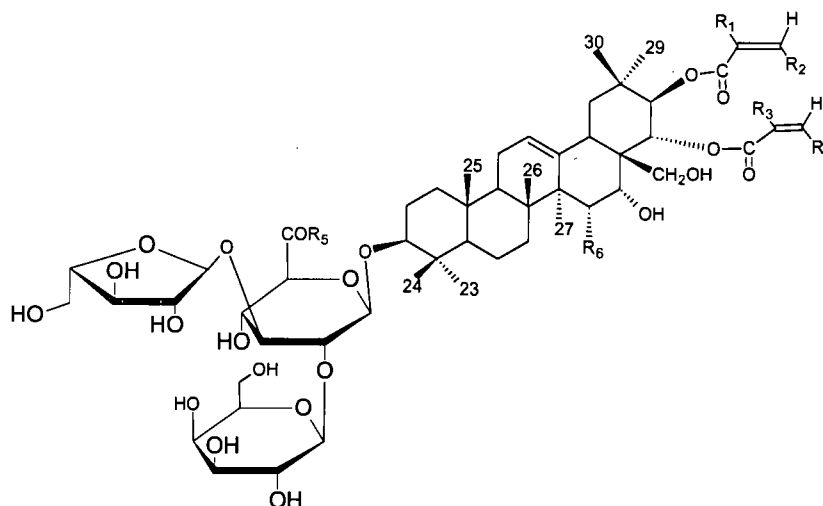
What is claimed is:

1. A compound selected from the group consisting of:



or a salt, ester, derivative or metabolite thereof.

2. A compound comprising the formula of (1):

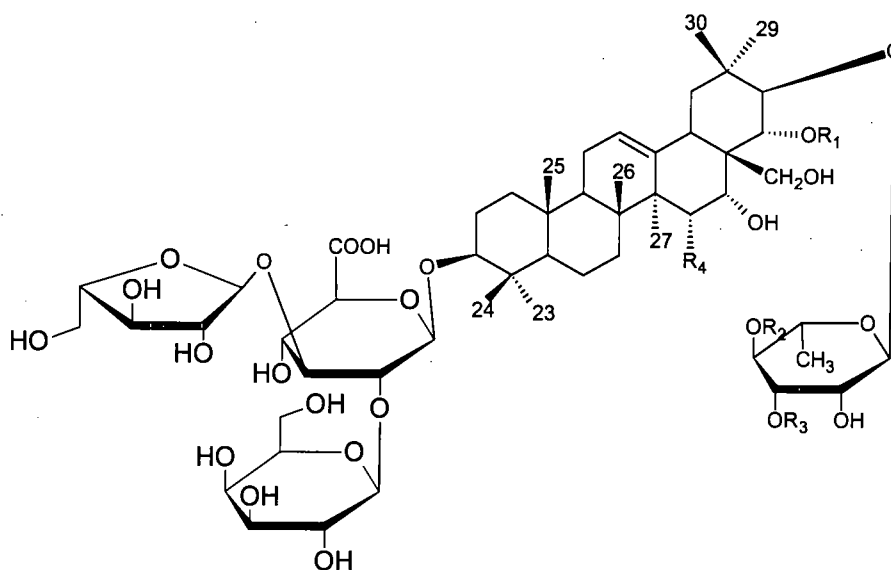


or a salt, ester, derivative or metabolite thereof,

wherein R₁, R₂, R₃ and R₄ comprises short aliphatic chain; and wherein R₅ and R₆ comprises an oxyl group.

3. The compound of claim 2, wherein R₁, R₂, R₃ and R₄ comprises CH₃; wherein R₅ comprises OH; wherein R₆ comprises H or OH; and wherein position 23-27, 29 and 30 comprises CH₃ or CH₂OH or COOH.

4. A compound selected from the group consisting of:



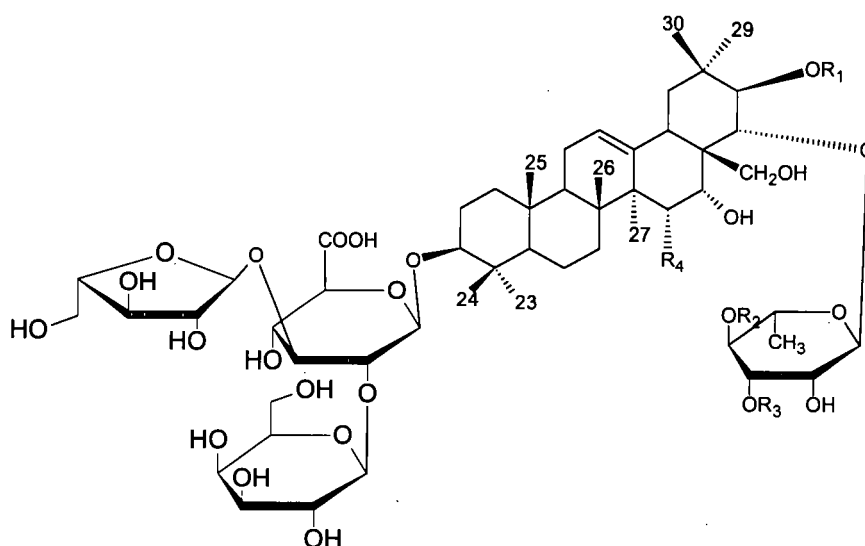
Wherein R1=A or B or C; R2=A or B or C; R3=A or B or C; R4=H or OH; A=angeloyl group; B = acetyl group; C= Hydrogen; and Positions 23-27, 29, 30=CH3 or CH2OH or COOH,

	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

; and



Wherein R1 =A or B or C; R2 =A or B or C; R3 =A or B or C; R4 =H or OH; A=angeloyl group; B = acetyl group; C= Hydrogen; and Positions 23-27, 29, 30 =CH3 or CH2OH or COOH

	R1	R2	R3
1	A	A	A
2	A	A	B
3	A	A	C
4	A	B	A
5	A	B	B
6	A	B	C
7	A	C	A
8	A	C	B
9	A	C	C

10	B	A	A
11	B	A	B
12	B	A	C
13	B	B	A
14	B	B	B
15	B	B	C
16	B	C	A
17	B	C	B
18	B	C	C

19	C	A	A
20	C	A	B
21	C	A	C
22	C	B	A
23	C	B	B
24	C	B	C
25	C	C	A
26	C	C	B
27	C	C	C

or a salt, ester, derivative or metabolite thereof.

5. A compound comprising the chemical formula:

3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene.

6. A compound comprising the chemical formula:

3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnopyranosyl-22-O-acetyl-3 β ,16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene

7. A method for identification of therapeutic compounds from *Xanthoceras sorbifolia* comprising steps of:

- (a) Extracting *Xanthoceras sorbifolia* powder with an appropriate amount of an organic solvent for an appropriate amount of time to form an organic extract;
- (b) Collecting the organic extract;
- (c) Refluxing the organic extract with an organic solvent for an appropriate amount of time to form a second extract;
- (c) Removing the organic solvent from the second extract;
- (d) Drying and sterilizing the second extract to form a *Xanthoceras Sorbifolia* extract;
- (e) Characterizing the *Xanthoceras sorbifolia* extract using HPLC chromatography;
- (f) Separating the *Xanthoceras sorbifolia* extract into components based on the results of the HPLC chromatography;
- (g) Screening each component for cytotoxicity;
- (h) Analyzing each component using cell activity assays to determine the active components; and
- (i) Purifying the active components; and
- (j) Determining the chemical structure of the compounds in the active components by NMR analysis.

8. A method for identification of therapeutic compounds from *Xanthoceras sorbifolia* comprising steps of:
- (a) Obtaining a *Xanthoceras Sorbifolia* extract;
 - (b) Characterizing the *Xanthoceras sorbifolia* extract using HPLC chromatography;
 - (c) Separating the *Xanthoceras sorbifolia* extract into components based on the results of the HPLC chromatography;
 - (d) Screening each component for cytotoxicity;
 - (e) Analyzing each component using cell activity assays to determine the active components; and
 - (f) Purifying the active components; and
 - (g) Determining the chemical structure of the compounds in the active components by NMR analysis.
9. The method of claim 7, wherein the *Xanthoceras sorbifolia* powder is obtain from the husk, branch, stem, leave, kernel, root, bark or seed shell of the *Xanthoceras Sorbifolia* herb.
10. The method of any one of claims 7 or 8, wherein the organic solvent is ethanol, methanol, ether, chloroform, alcohol or acetone.
11. The method of any one of claims 7 or 8, wherein the ratio of the *Xanthoceras sorbifolia* powder to the organic solvent is ratio of 1:2.
12. The method of claim 7, wherein the extraction step in (a) is performed 4-5 times for 20-35 hours each time.
13. The method of claim 7, wherein the refluxing step (c) is performed 2-3 times.
14. The method of any one of claims 7 or 8, wherein the cell activity assay is MTT Assay.
15. A composition comprising extract of *Xanthoceras sorbifolia* comprising saponins.

16. A method of preparing an extract from *Xanthoceras Sorbifolia* comprising steps of:
 - i. Extracting *Xanthoceras Sorbifolia* powder with an appropriate amount of one or more organic solvent for an appropriate amount of time to form an organic extract;
 - ii. Collecting the organic extract;
 - iii. Refluxing the organic extract with an organic solvent for an appropriate amount of time to form a second extract;
 - iv. Removing the organic solvent from the second extract; and
 - v. Drying and sterilizing the second extract to form a *Xanthoceras Sorbifolia* extract powder.
17. The method of claim 16 wherein the *Xanthoceras Sorbifolia* powder is prepared from the husk, branch, stem, leave, kernel, root, bark or seed shell of the *Xanthoceras Sorbifolia* herb.
18. The method of claim 16, wherein the organic solvent is ethanol, methanol, ether, chloroform, alcohol or acetone.
19. The method of claim 16, wherein the ratio of the *Xanthoceras Sorbifolia* powder to the organic solvent is ratio of 1:2.
20. The method of claim 16, wherein the extraction step in (i) is performed 4-5 times for 20-35 hours each time.
21. The method of claim 16, wherein the refluxing step (iii) is performed 2-3 times.
22. A composition capable of inhibiting tumor or cancer cell growth comprising an effective amount of the extract of claim 16.

23. The composition of claim 22, wherein the cancer is ovarian cancer.

24. A composition of claim 22, wherein the cancer is breast cancer, leukocyte cancer, liver cancer, ovarian cancer, bladder cancer, prostate cancer, skin cancer, bone cancer or brain cancer.

25. A composition comprising the extract of claims 15 capable of:

curing enuresis; relaxing the bladder; improving the response of cerebral cortex and cerebellum; preventing people from sleeping in the extra deep level; improving the sensory stretch receptor in the bladder wall to give a collect signal to central nerves system; relaxing bladder from stress or nervous tension, aging, hyper-reflexia which creates urgency and frequency micturition; relaxing the bladder tissue which contracts due to Ach; inhibiting AChE; producing anti-inflammatory effect; inhibiting virus; habiting human immunodeficiency virus protease; decreasing cholesterol and triglyceride level in blood; preventing cerebral aging, improving memory, improving cerebral functions; curing enuresis, frequent micturition, urinary incontinence, dementia, weak intelligence and Alzheimer's disease, autism, brain trauma, Parkinson's disease; treating arthritis, rheumatism, poor circulation, arteriosclerosis, Raynaud's syndrome, angina pectoris, cardiac disorder, coronary heart disease, headache, dizziness, kidney disorder; treating impotence and premature ejaculation; improving the function of bladder; inhibiting bladder cancer cell growth; suppressing deep sleep; increasing wake up alert; modulating the release, break down and uptake of Antidiuretic hormone (ADH) and its receptors; modulating the secretion, break down and uptake of Adrenocorticotrophic hormone (ACTH) and its receptors; modulating the release, break down and uptake of 5-hydroxytryptamine and its receptors; modulating the release, break down and uptake of Acetylcholine (Ach) and its receptors; modulating the release, break down and uptake of Adrenaline (AD) and its receptors; modulating the release, break down and uptake of Dopamine (DA) and its receptors; modulating the release, break down and uptake of Norepinephrine (NE) and its receptors; preventing sleep paralysis; modulating the formation, release, break down and activity the neuropeptides and their receptors; curing cancer such as breast cancer, leukocyte cancer, liver cancer, ovary cancer, bladder cancer, prostate cancer and brain cancer; improving the functions of the lung and the

bladder; and curing bone cancer.