Abstract: "Process for obtaining an antineoplastic phytotherapeutic compound derived from an extract from the plant bidens alba and antineoplastic phytotherapeutic compound derived from an extract from the plant bidens alba", encompasses a new plant extract that has antineoplastic activity and the process for obtaining it; the plant extract vegetal, as well as its fractions, have efficacy in inhibiting by cytotoxicity the lineages K562, HL60 and Nalm6 in-vitro, presenting an accentuated effect as their concentration is increased; inhibition of the rate of cell proliferation through the reduction of 3H-Thymidine collection as their concentration is increased, demonstrating anti-proliferation action.
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"PROCESS FOR OBTAINING AN ANTINEOPLASTIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM A N EXTRACT FROM THE PLANT BIDENS ALBA AND ANTINEOPLASTIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT BIDENS ALBA"

FIELD OF THE INVENTION

The present Invention Privilege patent is in regard to a phytotherapeutic process derived from a dichloromethane extract of a new plant species in the family Asteraceae. More specifically, the present invention is related to the compounds present in an extract from a plant of the genus Bidens sp. The diterpenes, triterpenes, sesquiterpenes and polyacetylene compounds present in its dichloromethane extract present active substances with anti-cancer pharmacological properties.

BASIS FOR THE INVENTION

Cancer has shown itself to be one of the major causes of human death over the years, and this has not greatly changed in current time, so much so that cancer is a serious health problem around the world. Much research has gone into the prevention, treatment and cure of cancer, with the objective of increasing the survival time and improving quality of life for the patient (Williams & Wilkins 1999).

The name of the disease, according to Galen, is due to the similarity between the tumescent veins around an external tumor and a crab's legs (Sontang 1984). The term "cancer" comes from the Greek "karkinos," crab, used by Hippocrates for indicating any kind of proliferating or chronic tumor formation (Ewing 1948; Suteiffe & Duin 1992). Since antiquity, there have been reports of cancer and its treatment. In the Elbers papyrus (1500 B.C.) there is a record of use of arsenical unguent for treatment. Documents from Hindu and Persian literature of the same period demonstrate a rudimentary knowledge regarding the disease (Ewing 1948). Hippocrates (460-375 B.C.) compiled a series of descriptions on cancer of the skin, breast, uterus and internal organs.

In the V century B.C., the predominant treatment consisted of incision and cauterization. Herodotus relates that Democedes (520 B.C.) cured Atossa, wife of Darius Huystaspis, of breast cancer. Hippocrates successfully extirpated
a neck cancer. Leonidas of Alexandria (180 A.D.) was the first to break with Hippocratic theories, expanding treatment of breast cancer, by sectioning through healthy tissues and cauterizing, as recommended by modern surgery. According to Ewing (Williams & Wilkins 1999), only the instruments have changed.

Celsus (30 B.C.-38 A.D.) distinguished several types of cancer, among them that of the breast. Cato employed treatment with charcoal Pliny mentions various rudimentary remedies. Galen (131-203), creator of physiology and experimental pathology, consolidated the humoral theory, according to which a deficiency or excess of blood, mucus or bile constitute the basis of all disease. The humoral theory dominant for a thousand years, attributing the origin of cancer to an excess of black bile (Williams & Wilkins 1999 and Barnes & Noble 1992). Paracelsus (1493-1541) was the first to oppose the theory of an excess of black bile as the cause of cancer. Thus, various remedies such as arsenic began to be employed. Little was known about the body and diseases, but a diet rich in vegetables was recommended for avoiding internal cancers (Ewing 1948).

Medical conceptions regarding the disease of cancer have historically been defined by society, building a common view of the disease, which even today keeps many of those ancient theories. Beginning in 1652, with Rudbeck's discovery of the lymph nodes, several scientists began pointing to the lymph system as the cause of cancer (Ewing 1948). Fabricius (1537-1619) distinguished several inflammatory tumorations of cancer, drawing attention to the inefficacy of incomplete extirpation. Hysterectomies were performed and several remedies were tested at that time. Before Rudbeck, Fabricius had already recommended removal of the axillary lymph nodes in breast cancer cases, and had developed an instrument for that purpose (Barnes & Noble 1992 and Ewing 1948). Marco Aurelio Severinus (1580-1656) extirpated malignant breast tumors, while resecting axillary lymph nodes.

The invention of the microscope in 1529 allowed Malpighi (1661) to describe red blood cells and demythologize black bile. Countless theories as to the origin of cancer were developed during the XVII and XVIII centuries(cancerous toxin, viruses,
thickening of the blood, abnormal tissue movements, outflow of congealable lymph, excessive acid in the body, possible inoculation by endovenous route, side effect of recurrent infections, organization of secretory products) (Ewing 1948; Barnes & Noble 1992).

With the invention of the achromatic microscope in Paris (1824), a new area in investigating and understanding cancer was begun. Raspail, in 1926, demonstrated that tissue growth occurs because of multiplication of its constituent cells. Several authors during the period, including Virchow, Alibert, Dupuytren and Velpeau, disagreed over the origin of tumors and their dissemination routes. However, the main theory, also defended by Virchow, was that tumors at any location have a mesenchymal origin (Ewing 1948; Barnes & Noble 1992). Later, Remak demonstrated that tumors establish themselves in specific tissues. Thus, only epithelial cells could give rise to epithelial tumors. Waldeyer was responsible for demonstrating that all normal tissues can become cancerous. In the last decades of the XIX century, tumors were exhaustively studied and classified, according to origin, and their etiological bases were established (Ewing 1948).

In the XX century, knowledge of cancer and the development of effective treatment methods began to offer real chances for cure. The first publications of what would become the modern concept of cancer were by Wilhelm Waldeyer-Hartz (1836-1921), creator of the term chromosome. He recognized that cancer begins in normal cells that undergo alterations, and grow excessively, producing metastases by means of blood or lymph vessels or interstitial fluids. According to this author, some cancers may become generalized, but a cure is possible, if the disease is diagnosed at its outset (Barnes & Noble; 1992).

Cancer research and treatment developed rapidly during the XX century, particularly during the last 30 years. Contributions from various diagnostic methods were also important in making it possible to learn the extent of the disease, allowing a complete stay and establishment of an appropriate treatment plan for each patient.

Treatment of cancer is generally based on surgical resection of localized solid tumors, radiotherapy for tumors in patients lacking for whom there are no clinical conditions or technical possibilities for a complete resection, and
chemotherapy in cases where the tumors are not solid, or disseminated solid tumors, as well as forms of leukemia (Boyd & Paull 1995).

Chemotherapy has the objective of killing malignant neoplasias by employing chemical substances, isolated or in combination, and is a new mode for systemic treatment of oncological disease, more recent than surgery and radiotherapy. (Bonassa 1998). The first records of effective chemotherapeutic treatment arose at the end of the XIX century with the discovery of Fowler solution (potassium arsenate) by Lissaver (1885) Coley toxin (a combination of bacterial products) in 1890. The first satisfactory results with using hormones for treating carcinomas of the prostate and breast arose at the beginning of the 1940s. During 2nd World War alkylant agents and their therapeutic effects were discovered. In 1943, a German air attack destroyed an American mustard gas depot in Bari, Itakt, leading to intense myelodepression among a group of contaminated individuals. That occurrence drew the attention of a group of clinical pharmacologists in service with the Pentagon. Thus, with the intention of producing therapeutic results, the drug was administered to a patient with a malignant lymphoma, who had a significant tumoral regression, albeit short lasting. During the 1940s, Sidney Farber found some cases of temporary remission of Lymphoblastic Leukemia in children, with the use of aminopterine and also "during the 1950s the first antibiotics with anti-tumoral activity were identified" (Bonassa 1998).

Investigations are currently underway in discovering drugs that are less toxic and more active, with a better therapeutic rate. The majority of antineoplastic agents lack specificity, that is, do not destroy only tumor cells. Thus, they are toxic to healthy tissue. One may consider that there are four types of chemotherapy according to their objectives: neo-adjuvant chemotherapy, adjuvant chemotherapy, curative chemotherapy and palliative chemotherapy (Azevedo 1989; Murad & Katz 1996).

Neo-adjuvant chemotherapy is the first therapeutic weapon used before other loco-regional modes, such as surgery or radiotherapy. It is applied in patients with extensive tumors, with the objective of achieving a reduction in tumor mass. It thus makes subsequent radiotherapy more effective and allows surgery to be more conservative. There can thus be a reduction of the signs
and symptoms of the disease, by destroying probable micrometastases (Azevedo 1989; Pviurad & Katz 1996). This is high-dosage chemotherapy, habitually combined, administered during the pre or post-operative period with the objective of reducing the size of the tumor, making surgery possible (Caseiro et al. 1997).

Adjuvant chemotherapy underwent major development during the 70s and 80s, at which time the concept of multidisciplinary therapeutics was initiated; it is administered after surgical removal radiotherapy. The objective is destruction of micrometastases that are thought to be present or that are too small to be detected (Hogan 1990).

Curative chemotherapy the noblest therapeutic form in the area of general chemotherapy, since it is applied as a first-line therapeutic resource and not in a subsidiary manner. This mode of chemotherapy is the only therapeutic weapon that, alone, can cure certain neoplasias (Bonassa 1998).

Chemotherapy known as "palliative" intends to increase survival time and/or allow better quality of life (relief of symptoms such as pain and obstruction). It is necessary when there is spread of the oncological disease, and with it very varied results are obtained, according to the sensitivity of different histological types of tumors (Azevedo 1989).

The morbidity associated with chemotherapy drugs is still a significant obstacle. Discovery of antineoplastic drugs is the current aim of all oncologists and researchers. Discovery of new drugs with distinct antineoplastic action is necessary and urgent. Substances with activity detectable using in vitro methods are subsequently evaluated in laboratory tests in vivo. These pre-clinical stages determine their activity, specificity and collateral effects at the cellular and tissue level or in specific organs. Such a determination is vital for the decision to permit administration of such drugs to humans (Boyd & Paull 1995).

Some 60% of the antineoplastic drugs identified and approved during the 1990s originate from natural products. The majority of these medications was obtained from plants, with the rest being derived from animals or microorganisms. Natural sources are abundantly present, and offer the best possibilities for finding substances of oncological interest. Several chemotherapy drugs currently in use are
derived from natural plants: vinca alkaloids, isolated from the Rosy Periwinkle, etoposidea derived from *Podophyllum peltatum*, taxol isolated from the *Taxus brevifolia* tree.

It is estimated that, as of today, only 10% of all the plants on the planet (species of higher plants) have been submitted to systematic analysis to identify active substances. The extremely rich flora existing in Brazil represents a practically unexplored source of new drugs. (Wagner & Bladt, 1996).

Given the great number of tumors existing in medical practice, it has become necessary to research, locate, develop and introduce new and more efficient treatment modalities into the medical therapeutic arsenal, so as to offer real opportunities for controlling neoplastic cells to the growing number of patients with malignant diseases.

The family Asteraceae encompasses around 25,000 and is one of the largest in the world. Chemical substances, such as polyacetylene, sesquiterpenoids, diterpenoids, triterpenoids, flavonoids, coumarins, benzofurans and benzopyrenes are particularly abundant in some families of dicotyledons, being commonly found among the Asteraceae (Hertz 1996). At present, there are records of chemical compounds from 5000 species and around 7000 constituents that have been isolated and identified (Zdero & Bohlmann 1990).

Some of the numerous plant species from the Asteraceae family have had their phytotherapeutic compounds studied and their pharmacological activity verified *in vitro* and *in vivo*. As an example one may cite *Lychnophora ericoides*, Brazilian arnica, whose analgesic and anti-inflammatory properties have been verified by a team led by Doctor Noberto Peporine Lopes (2001).

Another genus of this family, the genus *Bidens* L., is sub cosmopolitan, occurring in North and South America, according to Sherff (1937) with 233 species distributed among temperate and tropical regions worldwide, with approximately 284 varieties and 148 forms.

*Bidens alba* (L.) DC. (Asteraceae, Heliantheae) is native to the eastern region of Mexico across to Florida and the Caribbean Islands and the Gulf Coast of Mexico at altitudes above 2000m under the influence of a humid tropical climate. Identification of this species occurring exclusively in the coastal
region of Brazil was originally reported by M. D. Moraes (1998) and M. Magenta (1999). Later, identification and confirmation of the presence of *Bidens alba* in Brazil as a new species, was performed by means of molecular and phytochemical data (Grombone-Guaratini et al. 2004). *Bidens alba* together with *B. pilosa* and *B. subaltemans* form a complex of species in the southeastern region of Brazil (Grombone-Guaratini et al. 2004).

Pharmacological and phytochemical studies performed in other species of the genus *Bidens*, such as *Bidens pilosa*, have demonstrated that different extracts present anti-microbial, anti-helminthic, protozoicide and anti-ulcerogenic properties (NTDounga et al. 1983; Bondarenko et al. 1985; Geissberger & Sequin 1991; 1999). The Patent JP 2003-1 15703, of Musashino Meneki, describes some compounds derived from specific extracts of *Bidens pilosa*.

Recently published studies characterize the species of *Bidens*, identifying and differentiating each one.

*Bidens alba*: Herb with 0.4-1.2m, erect stalk with quadrangular green to vinaceous decumbent dorsal, erect or decumbent; with radiated capitules; rayed flowers with white and reflexive flowers, 6 - 16mm, yellow tubular flowers of the disk, ca. 5.5mm, and cipsels 6 - 10mm, angular, backed, hispid, with predominantly two aristas. The species presents 48 chromosomes and characteristic composition of terpenes and polyacetylene (Grombone-Guaratini, M.T.; Silva, K., L., Semir, J., Solferini, V.N. & Trigo, J.R Biochemical and Systematic Ecology 2005. 33 (5): 479486) and also a characteristic banding patter when analyzed using isoenzymes as molecular markers (Grombone-Guaratini, M.T., Semir, J. & Solferini V. N. Biochemical Genetics. 2005 43 (9/10)

*Bidens pilosa*: Herb around 0.3-1.0m; tetragonal green to vinaceous stalk; radiated capitules with white ligulated flowers, rarely light salmon in color, ca. 5mm or discoid capitules with ligules absent; flowers of the disk ca. 5mm tubular yellow, and cipsels 5 - 11mm angular, with sparse trichomes, backed with predominantly 3 aristas. The species presents 72 chromosomes, a particular composition of terpenes (Grombone-Guaratini, M.T.; Silva, K.L, Semir, J., Solferini, V.N. & Trigo, J.R. Biochemical and Systematic Ecology 2005. 33 (5): 479-486).
Research carried out with plants of the genus *Bidens* has sought a scientific foundation for the why they are used in traditional medical practices, principally in Africa and the Amazon.

Geissberger & Sequin (1999) using aerial parts of *Bidens pilosa*, found the substance phenylheptatriyne to which they attribute strong anti-microbial action against gram-positive bacteria and weak activity against gram-negative bacteria.

Brandao et al. (1997) suggest that the phenyl acetylene compounds found in *Bidens pilosa* leaves and roots may be responsible for antimalarial activity observed *in vitro* and *in vivo*.

Tan, Dimo & Dongol. (2000) demonstrated that the dichloromethane extract of *Bidens pilosa* is an excellent protector of the gastric mucosa. Additionally, according to the authors, the extract presents cytoprotective action against irritant substances, probably mediated by the cytoprotective action of endogenous prostaglandins. These authors attribute such activities to the presence of flavonoids in *Bidens pilosa* leaves.

On the other hand, several patents have been granted for herbicidal activities and decontamination of soil pollutants using *Bidens alba*; see, by example, United States Patent 6,492,301; 6,280,500;6,302,942; 6,713,434. However, no patent registration exists for antineoplastic activity by the compounds extracted in a specific dichloromethane extract of *Bidens alba*.

Therefore, it would be interesting to recognize new plant extracts possessing antineoplastic and/or anticancerogenous activities, with the intent of discovering phytotherapeutic drugs that are non-toxic and possess effective cytotoxic action against cancer cells, resistant or not to other chemical and phytotherapeutic medications currently existing.

**BRIEF DESCRIPTION OF THE INVENTION**

The present invention refers to a dichloromethane extract of substances found exclusively in *Bidens alba* for which are described previously unpublished mechanisms for physiological action and the process of obtaining them.

The phytotherapeutic substances present in this extract present cytotoxic and cytostatic properties acting simultaneously in inhibiting the growth of neoplasias (cancer). More specifically, the active substances present in this extract
are in the class of terpenes (diterpenes, triterpenes and sesquiterpenes) and acetylenes (polyacetylenes).

As a principal advantage, we have the fact that drugs obtained from this extract have already been tested successfully as a cure for gastric ulcer and its toxic effects were minimal for the animals tested. Furthermore, it has already been shown that both the raw extract and the fractions inhibit growth of neoplastic cells in-vitro.

BRIEF DESCRIPTION OF FIGURES

The present patent will be described in detail based on the figures listed below, in which:

- figure 1 illustrates analysis using CG-IN of raw extract of DCM, for this, observing the following parameters: Chromatographic conditions = column HP5, Temperature program 60°C — 3°C/min — 2000C, Injector at 250°C and detector at 280°C;
- figure 2A illustrates a photograph of the flower of Bidens alba;
- figure 2B illustrates a photo of the Karyotype of Bidens alba;
- figure 2C illustrates a photograph of the flower of Bidens pilosa;
- figure 2D illustrates a photo of the Karyotype of Bidens pilosa;
- figure 3 illustrates ccd analysis of the F1 fraction obtained from the CC of the DCM extract;
- figure 4 illustrates ccd analysis of the F2 fraction obtained from the CC of the DCM extract;
- figure 5 illustrates ccd analysis of the F3 fraction obtained from the CC of the DCM extract;
- figures 6A, 6B and 6C illustrate graphs where the results of the raw extract of Bidens alba are demonstrated, where the X-axis has different concentrations of the drug and the Y-axis represents the optic density of the aqueous extract, dichloromethane and the DMSO;
- figures 6D, 6E and 6F illustrate graphs where the growth inhibition effect (cytostasis) of the extract is demonstrated, where the X-axis shows different concentrations of the drug and the Y-axis represents the optic density of the aqueous extract, dichloromethane and the DMSO;
To verify the activity of the *Bidens alba* dichloromethane extract and chronic myeloid leukemia, sphenocytic iliichomi3 and acute lymphoid leukemia were employed.

**PROCESS FOR OBTAINING EXTRACT**

Preparation of the plant extract follows the following stages:

The fresh material (around 200.00 g) is placed over a period of 24 h in 2 l of distilled dichloromethane (CH$_2$C$_b$);

Later is vacuum filtered and dried with anhydrous sodium sulfate (200 grams);

Later the volume is reduced in a roto-evaporator without heating and under reduced pressure; and

The concentrate resulted in 2.3g of a solid dark substance;

That substance is solubilized in Tween 80® (Synth, Brazil).

**CHEMICAL ANALYSIS OF THE RAW EXTRACT AND ITS FRACTIONS**

The dichloromethane raw extract (DCM) of the medicinal plant *Bidens alba* was analyzed by chromatography in a fine chamber (ccd) and by gas chromatography coupled to a mass detector (CG-IN). The volatile components were analyzed by CG-IN and are presented in figure 1.

The CG-IN analysis of the DCM raw extract confirmed the presence of molecular ion m/z 204 that eluted between t$_r$ (retention time) 21.00 and 30.00 min. The majority sesquiterpenic components, t$_r$ 21.09 min (1.8%), t$_r$ 24.69 min (6.9%) and t$_r$ 27.41 min (24.4%), although presenting molecular ion m/z 204 were not identified by the Wiley library of the CG-IN; therefore, one may conclude only that they belong to the sesquiterpenic hydrocarbonate chemical class. An oxygenated sesquiterpene was also identified in t$_r$ 35.49 min (1.3%) of molecular ion m/z 220. The component of t$_r$37.08 min (55.8%) presented molecular ion m/z 164, and this suggests that it is the compound 1-phenyl-hepta-1,3,5-triino, characteristic of the species being studied.

The DCM extract was fractioned in a chromatographic column of eluted silica gel with solvent gradient (hexane, acetate, methanol) supplying 86 fractions that were combined through monitoring by ccd.
figures 7A, 7B, 7C and 7D illustrate graphs where the results of fractions F2 and F3 of *Bidens alba* are demonstrated;

figures 7E, 7F, 7G and 7H illustrate graphs where the growth inhibition effect (cytostasis) of fractions F2 and F3 is demonstrated;

figure 7I illustrates a graph where the results of fraction F1 of *Bidens alba* are demonstrated;

figure 7J illustrates a graph where the growth inhibition effect (cytostasis) of fraction F1 is demonstrated, where the X-axis shows different concentrations of the drug and the Y-axis represents the CPM (counts per minute) of leukemias K562 and HL60;

figure 7K illustrates a graph that demonstrates the growth inhibition effect (cytostasis) of the extract tested, where the X-axis shows different concentrations of the drug and the Y-axis represents the CPM (count per minute) of the PC3 cells under the effect of fraction F1 (TG1);

figure 7L illustrates a graph that demonstrates the effective results of fraction F1(TG1);

figure 7M illustrates a graph where it is possible to observe a phylogenetic distance between species of *Bidens*; and

figure 7N illustrates a graph where it is possible to observe as chemical differences between species of *Bidens*.

DETAILED DESCRIPTION OF THE INVENTION

*Bidens alba* is a plant with a short life cycle (2-3 years), whose life form varies from herb to short bush (0.4-1.2m); erect stalk with quadrangular green to vinaceous decumbent dorsal, erect or decumbent; with radiated capitules; rayed flowers with white and reflexive flowers, 6 - 16mm, yellow tubular disk flowers, ca. 5.5mm, and cipsels 6 - 10mm, angular, backed, hispid, with predominantly two aristas (Grombone-Guaratini et al. 2004).

Despite being part of the family Asteracea, its phylogenetic distance from species with similar phytotherapeutic activities is considerable (figure 7M). We may therefore assume that they possess distinct chemical substances, as may be demonstrated in figure 7N.
Below are presented the ccd analyses of fractions sent for assay of anticancer activity (figures 3, 4 and 5).

In all analyses the first point of application refers to the DCM extract, employed as a reference.

The DCM extract was fractioned in a chromatographic column of eluted silica gel with solvent gradient (hexane, acetate, methanol) supplying 86 fractions that were combined through monitoring by ccd.

With the data obtained by CG-IN on may conclude that the DCM extract studied contains mostly sesquiterpenic hydrocarbonates with a molecular weight of 204 (u.m.a.) and a minority oxygenated sesquiterpene (PM 220, t_r 35.49 min).

One may also observe a compound at t_r 37.08 min, molecular weight 164, that may be the 1-phenil-hepta-1,3,5-triino, characteristic of the species being studied.

When we cite sesquiterpene hydrocarbonates we include a list of the diterpenes, triterpenes, sesquiterpenes, polyacetylene and flavonoids of a dichloromethane extract present in *Bidens alba*.

In relation to the samples tested, analysis using ccd allows us to observe that the fractions presented distinct polarities in silica gel; however, information regarding components of those fractions may only be obtained through analysis using CG-IN and/or NMR spectroscopy (Nuclear Magnetic Resonance).

**TECHNIQUES USED FOR ASSESSING EFFICIENCY**

The cells utilized for verifying cytostatic activity (inhibition of cell proliferation) and cytotoxic activity (induction of cell death) are as leukemia lineages K562 (bone marrow; chronic myelogenic leukemia - Koeffler HP, Golde DW - 1980), HL60 (acute promyelocytic leukemia - Gallagher R, et al. 1979), Nalm6 (human B cell precursor leukemia - Hurwitz et al., 1979), KG1 (acute myelogenous leukemia - Kbeffler HP, Golde DW, R 1980) and Jurkat (acute T cell leukemia - Schneider et al., 1977) supplied by the American Type Culture Collection. Anti-proliferation activity was observed with the [3H] thymidine technique (H. R. Mauer - 1981) and cytotoxic activity with the MTT assays (Mosmann, T - 1983).

The cell lineages utilized in the tests were cultivated using RPMI medium (Sigma R5382) enriched with 10% FCS (Fetal Calf Serum Gibco catalogue
code: 16000-044), maintained at an atmosphere of 95% air and 5% CO2 at 37°C. For the cytostasis experiment, the cells were incubated with 1µCi of [methyl-³H]Thymidine (Amershambioscences - TRK120-5MCl) by plate during 18 hours. Collection of 3H-Thymidine was determined by counting in a liquid scintillation device (Liquid Scintillation Analyzer 2100TR - Packard Bioscience Company). To verify cytotoxic action, that is, to know if the drug was affecting cellular viability, we used the technique known as MTT (Mosmann, T. 1983), where the cells, after contact with the drugs being tested, are submitted to the tetrazolium soluble salt ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] MTT - Sigma M2128) and the viable cells are quantified colorimetrically using spectrophotometry (BioTek - PW XS).

RESULTS

The results of the raw extract of Bidens alba, demonstrated in figures 6A, 6B and 6C, have efficacy inhibiting in-vitro by cytotoxicity the lineages K562, HL60 and Nalmβ respectively, presenting an accentuated effect as drug concentration is increased. The growth-inhibiting effect on this same sequence of leukemia cells (cytostasis) of the extract tested is demonstrated in figures 6D, 6E and 6F. An inhibition of the rate of cell proliferation due to the reduction of 3H-Thymidine collection is demonstrated as the drug concentration is increased, suggesting anti-proliferation action.

The results of fractions F2(TG2) and F3(TG3) of Bidens alba, demonstrated in figures 7A, 7B, 7C and 7D, has in-vitro efficacy, inhibiting by cytotoxicity the lineages K562, HL60 Jurkat and KG1 respectively, presenting an accentuated effect as drug concentration is increased. The growth-inhibiting effect in the same sequence of leukemia cells (cytostasis) of this fraction is demonstrated in figures 7E, 7F, 7G and 7H. An inhibition of the rate of cell proliferation due to the reduction of 3H-Thymidine collection is demonstrated as the drug concentration is increased, suggesting anti-proliferation action.

The results of fraction F1(TG1) of Bidens alba, demonstrated in figure 7I show efficacy, inhibiting by cytotoxicity the lineages K562, HL60 in-vitro, presenting an accentuated effect as drug concentration is increased. The growth-inhibiting effect (cytostasis) of the extract tested is demonstrated in figure 7J. An inhibition of the rate of cell proliferation due to the reduction of 3H-
Thymidine collection is demonstrated as the drug concentration is increased, suggesting anti-proliferation action.

The results contained in figure 7L demonstrate the efficacy of fraction F1, inhibiting by cytotoxicity lineage PC3 in-vitro, presenting an accentuated effect as drug concentration is increased. The growth-inhibiting effect (cytostasis) of the extract tested is demonstrated in figure 7K. An inhibition of the rate of cell proliferation due to the reduction of 3H-Thymidine collection is demonstrated as the drug concentration is increased, suggesting anti-proliferation action.

The Bidens alba dichloromethane extract and its fractions were a powerful cytotoxic and cytostatic agent on neoplastic cells neo-plasias. To illustrate this affirmation erythrocytic, myeloid and lymphoid leukemia lineages and a prostate adenocarcinoma lineage were used.

The advantages from this extract and those fractions may be related to the discovery of new and more potent phytotherapeutic compounds, presenting fewer side effects and serving as an alternative to conventional chemicals found on the market, as well as reducing the cost of cancer treatment.

The description of the present invention described above was presented for the purposes of illustration and description. Beyond that, the description does not intend to limit the invention to the form revealed herein. As a consequence, variations and modifications compatible with the aforementioned descriptions, and the skill or knowledge of the relevant technique, fall within the scope of the present invention.

The modalities presented above try to better explain the known modes for use of the invention and to allow technicians in the field to utilize the invention in their or other modalities, and with various modifications necessary for specific applications or uses of the present invention. The intention is that the present invention shall include all modifications and variations of the same, within the scope described in the report and in the appended claims.
CLAIMS

1. "PROCESS FOR OBTAINING AN ANTINEOPLASIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT *BIDENS ALBA*", characterized by the fact that preparation of the plant extract occurs by means of the following stages: fresh leaves of *Bidens alba* are placed for around 1 to 100 hours in a sufficient quantity of distilled dichloromethane; later that material is vacuum-filtered and dried with anhydrous sodium sulfate; later the volume is preferably reduced in a roto-evaporator without heating and under reduced pressure; a concentrated extract is obtained; that concentrate is solubilized in detergent.

2. "PROCESS FOR OBTAINING AN ANTINEOPLASIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT *BIDENS ALBA*", according to claim 1, characterized by leaves of *Bidens alba* being preferably placed for around 10 to 50 hours in a quantity of 1 to 5 liters of dichloromethane, varying according to the substrate.

3. "PROCESS FOR OBTAINING AN ANTINEOPLASIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT *BIDENS ALBA*", according to claim 1, characterized by the fact that the detergent used for solubilizing the concentrated extract is preferably a non-ionic detergent.

4. "ANTINEOPLASIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT *BIDENS ALBA*", characterized by the fact of presenting a *Bidens alba* extract with antineoplastic activity in both its raw concentration and its fractions and a sufficient quantity of a pharmaceutically acceptable vehicle.

5. "ANTINEOPLASIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT *BIDENS ALBA*", as claimed in 4, characterized by the fact of foreseeing the existence of, at least three fractions, F1, F2, and F3 that are obtained through fractioning of the raw extract, in a chromatographic column solvent gradient, or other technique capable of separating these three fractions.

6. "ANTINEOPLASIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT *BIDENS ALBA*", as claimed in 4,
characterized by the fact that the raw extract contains mostly sesquiterpenic hydrocarbonates, um minority oxygenated sesquiterpene and/or flavonoids.

7. "ANTINEOPLASTIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT BIDENS ALBA", as claimed in 4, characterized by the fact of that the raw extract and its fractions are capable of inhibiting by cytotoxicity leukemia lineages, such as K562, HL60 and Nalmδ, presenting an accentuated effect as their concentration is increased and capable of inhibiting the rate of cell proliferation through the reduction of 3H-Thymidine collection as their concentration is increased, demonstrating anti-proliferation action.

8. "ANTINEOPLASTIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT BIDENS ALBA", as claimed in 4 a 7, characterized by the fact of that as fractions F2 (TG2) and F3 (TG3) have efficacy in inhibiting by cytotoxicity the lineages K562, HL60 Jurkat and KG1 in-vitro, presenting an accentuated effect as their concentration is increased; the F1 fraction (TG1) has efficacy, inhibiting by cytotoxicity the lineages K562, HL60 in-vitro, presenting an accentuated effect as their concentration is increased; as fractions F1, F2 and F3 present an inhibition in the rate of cell proliferation through the reduction of 3H-Thymidine collection as their concentration is increased, demonstrating anti-proliferation action.

9. "ANTINEOPLASTIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT BIDENS ALBA", as claimed in 4, characterized by the fact of foreseeing the manufacture of new pharmacological compounds directed towards antineoplastic activity.

10. "ANTINEOPLASTIC PHYTOTHERAPEUTIC COMPOUND DERIVED FROM AN EXTRACT FROM THE PLANT BIDENS ALBA", as claimed in 4 and 9, foresees the manufacture of pharmacological compounds characterized by the fact of using, as their active principle, Bidens alba extract, or its fractions.