In Vitro Cancer Research on Ancient Herbal Remedies: A Changing Trend

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ABSTRACT

Since cancer is one of the leading causes of death worldwide, accounting for 13% of all deaths, there is a global demand for research to develop new treatments. In search of new cancer preventive and treatment agents, hundreds of plant extracts and compounds have been evaluated for their potential to inhibit cancer cells in vitro, many of which have been used for centuries by native populations from specific geographic regions around the world. Some ancient remedies have now been shown to inhibit the proliferation of specific cancer cells by having a desirable effect at the molecular level. More recently, the number of in vitro gene studies to understand the effects of these herbal medicines on various forms of cancers has increased due to the advancement in molecular techniques. Improved research methods and increased knowledge of the effects of plant extracts on cancer genes is allowing individualistic diagnoses, prognoses, and treatments.

Keywords: cancer treatment, herbal remedies, single-nucleotide polymorphisms, traditional Chinese medicine.

INTRODUCTION

Various herbs from across the world have been used for millennia as traditional medicines for cancer and other ailments. In some cases, the same plant found in different isolated regions was used for similar treatments. For example, Psidium guajava L. and Ocimum basilicum L. have both been used in Brazil and Thailand to treat diseases (Holetz et al., 2002; Manosroi et al., 2006; Vieira and Simon, 2000). Before modern times, the effects of plant-based treatments were typically evaluated by changes in signs and symptoms. The methods of approaching cancer research and treatment today have changed dramatically since the trial and error of ancient times. More recently, at both the cellular and genetic levels, modern in vitro technologies have provided a more comprehensive scientific approach for understanding not only whether a particular remedy might work but often insight into the mechanism(s) by which the plant’s chemical components have their effects. This has made it possible to examine ancient and traditional medicines to determine their suitability and potential use against diseases in the present era.
In recent years, in vitro studies have been performed on a select number of ancient herbs. In some cases, extracts or compounds of these traditional medicines have been shown to have a potent effect on the proliferation of specific cancer cells by influencing changes in molecular activity and gene regulation. These findings are opening new lines of investigation for symptom reduction and cancer cell elimination. Specific gene mutations have been directly linked to increased risks of certain cancers which may vary based on the patient’s ethnicity (Ruiz-Marvazet et al., 2010; Yeager et al., 2007; Stacey et al., 2007). Determining the specific set of genes that is altered after cancer is diagnosed and understanding the effects of herbal extracts on the gene mutations may provide a more individualistic prognosis and lead to an improved understanding of treatment options.

Modern Work on Ancient Herbal Remedies from Different Regions

The ancient and modern use of traditional herbal remedies is documented from all regions of the world. China is represented in traditional medicine more than any other region and many of those remedies have now been scientifically investigated and published in scientific journals. In other regions, there are fewer scientific publications of ancient herbal remedies, such as in African countries. A sampling follows on traditional herbs from various geographic regions that have been tested and published in the literature.

East Asia

Many ancient remedies around the world have been shown to exhibit antiproliferative effects on cancer cells (Table 1), with the majority coming from China and other parts of East Asia. One example is Paeoniae radix, commonly known as red peony, which has been used to treat liver diseases for centuries in China without any explanation of an underlying mechanism for its apparent activity. Recently, it was shown that the human hepatoma cell lines HepG2 and HepG3B were inhibited after exposure to P. radix by the induction of apoptosis through a pathway independent of tumor suppressor gene, p53 (Lee et al., 2000). The investigators concluded that this occurred by induction of apoptosis through a pathway independent of the tumor-suppressor gene, p53.

Another example of an ancient Chinese herbal remedy is the aqueous solution of Astragali radix, commonly known as milk vetch. Extracts of this herb have been tested against the growth of gastric cancer cell lines AGS and KATO-III, colon cancer cell line HT29, breast cancer cell line MDA-231, and melanoma cell lines MEL7 and MEL14 (Lin et al., 2003). The greatest inhibitions of cell proliferation were associated with the gastric cancer cell lines, with 68% and 62% inhibitions of AGS and KATOIII, respectively. The reduction of the growth of AGS cells was shown to be concentration and time-dependent and was found not to be due to apoptosis (Lin et al., 2003). Panax ginseng is another plant that has been used as traditional Chinese medicine and is now found frequently in Korean, Japanese, and Chinese prescriptions for chronically ill patients (Chang et al., 2003). Three of eleven saponins recently isolated from P. ginseng were found to effectively inhibit cell proliferation of glioma, lung, prostate, breast, and pancreatic cancer cells (Wang et al., 2007). This study further indicated that the inhibition of lung cancer cell proliferation occurred through apoptosis and cell cycle arrest and that the number of sugar moieties, variation in substituent groups, and the type of dammarane each impact the anticancer activity.

Another example of an ancient herbal remedy still being used is Aegle marmelos, a medicinal plant from Bangladesh that has demonstrated inhibitory activity on the proliferation of a variety of cancer cell lines (Lampronti et al., 2003). Among the cell lines tested were leukemic cell line K562, T-lymphocyte Jurkat cells, MCF-7 breast cancer cells, and melanoma Colo38 cells. Three extracts obtained from A. marmelos showed the greatest antiproliferative effects on K562 leukemia cells, although all cell lines tested were inhibited by these extracts in a dose-dependent manner. Mixtures of traditional medicinal herbs from East Asia have also been used in the treatment of cancer. For example, PC-SPES is a commercially available mixture of 8 herbs: chrysanthenum, licorice, isatis, scutellaria, saw palmetto, Panax pseudo-ginseng, Rabdosia rubescens, and Ganoderma lucidum (DiPaola et al., 1998). The ingredients in this mixture have been used in Chinese medicines with some scientific research to support its use (Kitagawa, 2002; Sonoda et al., 2004). For example, P. pseudo-ginseng (pseudoginseng or mountain paint) has been used for centuries in China as an herbal medicine and has recently been found to have antioxidant activities (Chan and Tomlinson, 2000). G. lucidum (lingzhi mushroom) has been used in Chinese medicines to treat disorders of the immune system and has been shown to inhibit proliferation of human colorectal cancer cells (Xie et al., 2006). R. rubescens (blushed rabdosia), an herbal remedy commonly used in China, has been used for its anticancer activity against esophageal cancer (Chen et al., 2005). It was found that in patients with prostate cancer, PC-SPES decreases testosterone levels during use and that the levels increase 3 weeks after use is discontinued (DiPaola et al., 1998). In all 8 patients who participated in the study, PSA (prostate specific antigen) levels had a concomitant decrease after the first treatment of PC-SPES. The concentration of PSA increased within 3 weeks after the treatment was halted. Other studies have reported that PC-SPES inhibits the growth of MCF-7 cells in vivo (Halicka et al., 1997).

In addition to herbal extracts, specific compounds isolated from herbs, and mixtures of herbs, oils from plants have also been tested for their cancer fighting properties (Manosroi et al., 2006). One study tested 17 Thai medicinal plants against human mouth epidermal carcinoma KB and murine leukemia P388 cell lines. While the majority of the plants tested had IC_{50} well below 1 mg/ml, oil from leaves of Psidium guajava L., guava, showed the greatest antiproliferative effect against the KB cell line (IC_{50} = 0.0379), while oil from Ocimum basilicum L. (sweet basil) had the greatest antiproliferative effect against the P388 cell line (IC_{50} = 0.0362). Interestingly, both plants have also been used in Brazilian folk medicine to treat infectious diseases (Holetz et al., 2002; Vieira and Simon, 2000).
Middle East

One of the most widely used herbs for medicinal purposes in the Middle East for millennia is the Ficus species (Lansky et al., 2008). Commonly known as fig, several compounds have recently been extracted from the stems of F. formosana f. formosana (Moraceae) and have been shown to have cytotoxicity activity against HepG2, PLC/PRF/5 hepatocellular, and Raji lymphoblastoid cancer cells in vitro (Bisi-Johnson, 2011). In addition, triterpenes were extracted from F. microcarpa roots and eleven were tested against the proliferation of HONE-1 nasopharyngeal, KB oral epidermoid, and HT29 colorectal carcinomas, with eight having IC\textsubscript{50} values ranging from 4.0–9.4 µM (Chiang et al., 2005). In other work, 6-O-acetyl-B-D-glucosyl-B-sitosterols were isolated from F. carica and tested against various cancer cell lines (Rubnov et al., 2001). At concentrations of 50 µg/ml, DJ-75 Burkitts lymphoma cells and Jurkat T-cell lymphoma cells were inhibited at levels of 87% and 81%, respectively. At concentrations of 25 µg/ml, Jurkat and DU-145 prostate cancer cells were inhibited by 62% and 61%, respectively. Each of these studies validates the use of Ficus species in traditional medicine by Middle Eastern and other populations.

Allium sativum, commonly known as garlic, has been one of the most widely used herbs across Jordan since it is viewed as a safe medicinal plant (Aburjai et al., 2007). A manganese superoxide dismutase has been isolated from this herb with inhibitory effects on the growth of porcine endothelial cells and B16 mouse melanoma cells, in vitro (Sfaksi et al., 2009). Ajoene is another active compound isolated from A. sativum that has been tested against MCF-7, KB, Bel 7402 hepatocellular, BGC 823 gastric, HCT colon, HL60 promyelooleukemic, and HeLa cervical cancer cells (Li et al., 2002). IC\textsubscript{50} values ranged from 5.2 µM to 26.1 µM, with HL60 cells having the lowest value. Furthermore, an in vivo study determined the activity of aged garlic extract against the MBT2 murine bladder tumor model with significant inhibitions of tumor growth after exposure to doses of 50 and 500 µg/ml and improvement in survival seen in the latter (Lamm and Riggs, 2000). Collectively, these studies support further in vivo research involving A. sativum in preventing or treating different forms of cancer in humans.

Silybum marianum, milk thistle, has been used for centuries in the Middle East, as well as in Europe, to treat gall bladder and liver ailments (Rubnov et al., 2001). Silymarin is a flavonolignan from S. marianum, which has been found to interfere with cell cycle regulators and apoptotic proteins (Ramasamy and Agarwal, 2008). It inhibits epidermal growth factor receptor (EGFR) signaling along with suppressed expression of cyclin-dependent kinase (CDK). There is a coinciding upregulation of the CDK inhibitors, CDK-interacting protein 1 (p21) and CDK inhibitor 1B (p27), and growth arrest at G1 and G2. The presence of silymarin leads to apoptosis through the mitogen-activated protein kinase (MAPK)/C-JUN N-terminal kinase (JNK) pathway. Silibinin, the most active compound of silymarin, has been shown to interfere with metastasis (Ramasamy and Agarwal, 2008; Kim et al., 2009).

Europe

Although not as widespread as in Asia and the Middle East, there is evidence indicating prevalence in the use of ancient herbal remedies to treat cancer among Europeans. Extracts of Viscum album, widely known as mistletoe, are among the most prescribed medicinal herbs in Europe (Kienle and Kiene, 2010). An aqueous extract of V. album was investigated for its cytotoxic effect against Jurkat cells. Enhanced cytotoxic effects have been reported when V. album was combined with doxorubicin (Sabova et al., 2010). It has been shown that aqueous extracts of this herb exhibit a concentration-dependent cytotoxicity on bladder cancer cells (Hunziker et al., 2009). Another study has reported that V. album coloratum agglutinin is effective at eliminating the human colorectal cancer cell line COLO in a time and dose-dependent manner by apoptosis as shown through caspase-8 activation (Khiil et al., 2007).

Arayne et al. (2007) and Ellis (2005) reported that Berberis vulgaris (pepperidge bush) and Coptis chinensis (goldthread) have played important roles in a wide variety of health

![Table 1: Examples of herbs/plants from different region of the world with anticancer activity.](https://example.com/table1.png)
problems for centuries across Europe and North America and also in China, respectively. Berberine is an isoquinoline alkaloid extracted from both genera, Berberis and Coptis, which was reported to be a Cyclooxygenase-2 (COX-2) inhibitor, thereby restraining the growth of DLD-1 colon cancer cells (Fukuda, 1999). The transcription of the COX-2 gene, which codes for the enzyme that plays an important role in the tumorigenesis of colon cancer cells, is also suppressed. It had previously been reported that the cyclooxygenase-2 (COX-2) enzyme, enhanced by the expression of oncogenes such as sarcoma viral oncogene homolog (V-SRC), Harvey rat sarcoma viral oncogene homolog (V-HA-RAS), and wingless-type MMTV integration site family (WNT), is activated in colon cancer cells, resulting in an increase in prostaglandins (Sheng et al., 1997). It has been proposed that berberine, through the COX-2 inhibition mechanism, could also help to inhibit breast cancer cells (Howe et al., 2001).

**Americas**

Uncaria tomentosa, a species of Rubiaceae commonly called cat’s claw, has traditionally been used in South America for the treatment of inflammatory conditions, arthritis, and cancer (Kemper, 1999). Two extracts from the bark of this plant have been shown to inhibit the growth of MCF-7 cells up to 90% (Riva et al., 2001). In another study, sarcoma osteogenic human osteosarcoma (SAOS) cells, MCF-7 breast cancer, and HeLa cervical cancer cells had proliferation inhibited in a dose-dependent manner as well as increases in caspase 3 activity when exposed to two extracts of *U. tomentosa*, with HeLa cells being the most sensitive (De Martino et al., 2006). *U. tomentosa* has also been reported to exhibit a potent inhibitory effect on THP-1 human acute monocytic leukemia cells by inactivating TNF-α through NF-κB (Allen-Hall et al., 2010).

Fifty-one Brazilian plants were selected for leaf extraction and determination of activities against B16 mouse melanoma, HL-60 human leukemia, MCF-7, and HCT-8 colon cancer cells (Dos Santos Júnior et al., 2010). Of the extracts tested, those that displayed the strongest activity against the tumor cells were *Lantana fucata*, *Copaifera langsdorffii*, and *Momordica charantia*. Toxicity studies were performed on each of these to determine the impact on sea urchin development, brine shrimp, and hemolytic activity against mouse erythrocytes. Only *L. fucata* showed no toxicity in any of these studies, suggesting that it could potentially be the best choice in developing new anti-cancer drugs.

Native to North and South America as well as East Asia, *Phytolacca americana*, pokeweed, has been used by Native Americans as a laxative, to induce vomiting, and to treat inflammation, rashes, and breast problems (Jones, 2006). Pokeweed antiviral proteins (PAP) are single chain ribosome-inactivating proteins (RIPs) derived from *P. americana* that work by N-glycosidase activity to remove an adenine base from a conserved portion of the rRNA in eukaryotes and prokaryotes to inhibit translation. PAP has been conjugated with monoclonal antibodies, such as B43 and TXU, and the combinations have been found to be effective against B and T cell leukemias in mice, respectively (Uckun et al., 1986; Waurzyniak et al., 1997). In a recent study, Maness et al. (2012) found that extracts derived from *P. americana* inhibited the proliferation of HCT-116 colon cancer cells through activating caspases 3, 8, and 9.

**Africa**

Many traditional herbal remedies are used across Africa, although few have been reported scientifically in the literature. Found in arid areas of South Africa and Namibia, *Dicoma capensis* is a low-lying herb that has been used by the Khoisan people as a traditional medicine. This plant has recently been found to have cytotoxic effects against breast cancer cells. In a study by Steenkamp and Gouws (Steenkamp and Gouws, 2006), IC₅₀ values were found to be relatively low, 30 µg/ml and 31 µg/ml, when this plant was tested against MCF-7 and MCF-12A cancer cells, respectively. Other South African medicinal plants used for cancer treatment by traditional healers are *Bidens pilosa*, *Centella asiatica*, *Cnicus benedictus*, *Hypoxis hemerocallidea*, and *Sutherlandia frutescens*. When tested on cancer cells, none of these plants exhibited significant antiproliferative activity as much as that of *Dicoma capensis*.

The most commonly used plant in the Eastern Cape Province of South Africa was determined to be *Solanum aculeastrum* whereby the fruits are boiled, filtered, and the decoction administered daily (Koduru, 2007). Tomatidine and solasodine have been extracted from the berries of this herb and tested against HeLa, MCF7, and HT29 cells. The greatest antiproliferative effect was against HeLa cells when using both steroid glycosides combined, suggesting that this should be the choice for further testing regarding the effects of *Solanum aculeastrum* against cervical cancer cells (Koduru et al., 2007).

*Savalia* species have also been used as traditional medicines in Africa against cancer as well as inflammation, infections, malaria, and more (Shoemaker et al., 2005; Watt and Breyer-Brandwijk, 1962). Seventeen of these species were tested against MCF-7, HT-29 colon, and SF-268 glioblastoma cancer cells in vitro (Kamatou et al., 2008). It has been shown that *S. radula* was the most active against MCF-7 and SF-268 while *S. lanceolata* exhibited strong activity against HT-29 with IC₅₀ values as little as 9.69 µg/ml, 8.72 µg/ml, and 12.12 µg/ml, respectively. In one last example from Africa, six traditional medicinal plants used in South Africa were selected for methanol extraction and further ethyl acetate, butanol, and water fractionation (Bisi-Johnson et al., 2011). Each product was tested against Huh-7 human hepatocarcinoma cell line. The findings indicated that the methanol extract of *Eucomis autumnalis* had a lower IC₅₀ than the berberine positive control with 7.8 µg/ml compared to 9.8 µg/ml. The methanol extract of *Hypoxis latifolia* and the methanol extract and butanol fraction of *Cytalthula uncinulata* also had relatively low IC₅₀ values of 24.4 µg/ml, 24.8 µg/ml, and 30.0 µg/ml, respectively. The low IC₅₀ values warrant further testing for each of these extracts against hepatocarcinoma and other cancer cell lines.
Changing Trends in Approach to Cancer Testing and Treatment

Regarding the use of traditional herbal remedies that have been used for centuries such as those mentioned above, ancient populations had their own “scientific” approach involving simple trial and error (Nutton, 2004). The validity of treatments that our ancestors honored as they were passed down through generations primarily involved evidence of patients’ signs and symptoms. It is now beneficial to examine the possible effectiveness of those traditional medicines by modern technologies. In some cases, that substantiation has already been made. In cases where folk remedies are not supported by research, science can serve to cast doubt on “old wives’ tales” or folk remedies. Technological progress has allowed what began merely as trial and error or guesswork to be substantiated, or not, by scientific methods that include cellular, molecular, and DNA studies.

Tests at the cellular level have evolved from the MTT assay (Mossman, 1983) and flow cytometry (Clark et al., 1989; Reid et al., 2000) to the recent methodology of detecting rare isolating tumor cells using microchip technology (Arayne et al., 2007). Thus, there has been a movement from colorimetric tests that measure enzyme activity and analyzers that count cells by using scattered light to the direct detection of circulating tumor cells. In the latter approach, circulating tumor cells can be identified in blood samples of patients with lung, breast, pancreatic, colon, and prostate cancers 99% of the time using antibody coated microposts, and may replace or minimize the use of invasive biopsies (Nagrath et al., 2007).

Cancer markers can also be detected using polymer microchips which are capable of distinguishing between cancer types at the DNA level (Gulliksen et al., 2005). These microchips have been constructed to detect DNA variations known as polymorphisms using oligonucleotides (Tillib and Mirzabekov, 2001). DNA chip technologies vary by DNA fragment size, array method, and hybridization and detection methods. Single-nucleotide polymorphisms (SNPs) found in coding and noncoding regions, which can also be markers for cancer, can now be detected as well. Understanding specific genes involved in the development and progression of various cancer types is especially beneficial as research is helping to identify specific herbs that alter the expression of those genes. For example, shikonin is an ingredient in Lithospermum erythrorhizon that has been found to reduce the expression of steroid sulfatase (STS) genes in MCF-7 and SK-BR-3 breast cancer cells (Zhang et al., 2009). The down-regulation in STS gene expression helps decrease the synthesis of estrogen, which plays a role in the development and progression of breast cancer. In another example, the traditional Chinese herbal remedy known as Tian xian has been shown in human hepatocyte culture to increase the expression of CYP3A4, which is involved in drug metabolism (Lichti-Kaiser and Staudinger, 2008). Taken along with prescribed chemotherapeutic drugs, this Tian xian may be contraindicated. This combined approach of understanding the genes responsible for the cancer as well as herbs that may change the expression of those genes is changing the approach to cancer treatments. Identifying the specific SNP involved in each case of cancer will allow for more individualized cancer treatments since different herbs affect different cancer genes.

Discovering Herbs that Alter Expression of Specific SNPs May Lead to a More Individualistic Approach to Cancer Diagnosis, Prognosis, and Treatment

The presence of different SNPs is associated with increases in the risks of various types of cancer. For example, one study involving cases from Texas and Europe determined the top ten SNPs most associated with lung cancer (Amos et al., 2008). Those most associated with an increased lung cancer risk were rs1051730 and rs8034191, both belonging to the chromosome region 15q25 which contains receptors for nicotinic acetylcholine. Various herbal remedies that may be tested are likely to affect the various SNPs in a different manner. Herbs and other cancer treatments have already been shown to have different effects on the same cancer depending on the genes present. Several SNPs associated with increases in familial colorectal cancer have been found at 8q24.21 (Tomlinson et al., 2007). The most frequently associated SNP at this location was rs6983267. Similarly, a high-risk SNP for prostate cancer was found to be rs6983267 and located at locus 8q24 in patients of European origin (Yeger et al., 2007). Furthermore, studies have been carried out on estrogen-receptor positive breast cancer patients in Iceland and common variants found on 2q35 and 16q12 with SNPs rs13387042 and rs3803662, respectively (Stacey et al., 2007). It is likely that patients with the different high-risk SNPs, which usually vary according to ethnicity, will be affected differently by diverse herbal remedies.

For example, the polymorphisms mentioned above in breast cancer patients from an Icelandic origin differ from those found in both African-American and European populations. Two SNPs found to be associated with an increased risk of breast cancer in European women are rs4415084 and rs10941679 at locus 5p12 while the SNP most associated with an increased risk for African American women at that same locus was determined to be rs16901937 (Ruiz-Narvaez et al., 2010). Since there are different SNPs leading to the same cancer type, it is likely that various herbal extracts will affect that type of cancer in a dissimilar pattern, depending on the SNP- or gene combination- involved. Since polymorphisms vary among individuals with the same type of cancer, new technologies that allow a better understanding of the impact of herbal remedies on SNPs may lead to a more individualistic approach to cancer treatment. The candidate gene approach consists of identifying genes responsible for diseases and the new digital candidate gene approach has emerged since the world wide web offers numerous genomic resources (Zhu et al., 2010). This will allow physicians to better understand the primary cause of an individual’s cancer and to choose a suitable treatment that is most effective for that specific polymorphism since each variation likely responds to various drugs or alternatively, herbal remedies, differently. Similarly, the patient’s prognosis changes depending on the specific polymorphisms that lead to cancer.
For example, several genes may be mutated in colon cancers including APC, K-RAS, BCL-2, and RB-1 (Ceol et al., 2007; Kambata-Ford et al., 2007; Poincloux et al., 2009; Nevin, 2001). When BCL-2 function is lost in patients with stage II colon cancer, risk of relapse increases whereas when BCL-2 function is detectable, tumor growth is slower (Poincloux et al., 2009). Furthermore, it is widely regarded that APC gene inactivation is the initiating step in human colorectal cancer cell transformation (Silverman et al., 2002). In fact, in a study of colonic adenocarcinomas, 59% had frameshift mutations in the APC gene (Samowitz et al., 2007). Similarly, those with K-RAS mutations often have a lack of response to cetuximab, a common anticancer drug used in colorectal cancer treatment, and lower disease control rates than patients without the mutation (Kambata-Ford et al., 2007). Determining the statuses of each of these genes could benefit colon cancer patients. Preventing or reversing any of these mutations, potentially through yet untested ancient herbal remedies, could prove to be valid prevention/treatment options. As for RB-1, however, it has been suggested that it may not be useful at all in determining a prognosis in colorectal cancers (Poller et al., 1997).

CONCLUSION

A variety of studies have been performed to substantiate the widespread use of traditional herbal remedies that exist around the world. Whereas many of the herbal remedies previously mentioned have been studied at the cellular level and protein levels, studies involving the effects of these herbs on gene expression have more recently emerged. Current scientific trends will increase the number of studies that determine the specific genes that are affected in cancer cells after exposure to various plant extracts as well as whether the high-risk SNPs associated with the particular cancer are affected. This information will assist in the selection of specific herbal treatments that are better directed to target mutations involved in each cancer type.

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