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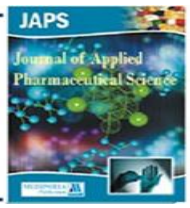

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In Vitro Cancer Research on Ancient Herbal Remedies: A Changing Trend

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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-Marvaez *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 Hep3B 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: chrysanthemum, licorice, isatis, scutellaria, saw palmetto, *Panax pseudo-gensing*, *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-gensing* (pseudogensing 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* (blushred 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).

Table. 1: Examples of herbs/plants from different region of the world with anticancer activity.

Region of Ancient Use	Herb Name	Cancer Cell Lines Affected	Reference
China	*Paeoniae radix*	HepG2, Hep3B hepatoma	Lee *et al*., 2002
China	*Astragali radix*	AGS, KATOIII gastric	Lin *et al*., 2003
China	*Panax ginseng*	H838, H358 lung	Wang *et al*., 2007
Bangladesh	*Aegle marmelos*	K562 leukemia	Lampronti *et a*l., 2003
China	*Rabdosia rubescens*	DU-145 prostate, MCF-7, A2780 and PTX10 ovarian	Chen et al., 2005
China	*Ganoderma lucidum*	SW 480 colorectal	Xie *et al*., 2006
Thailand	*Psidium guajava L.*	KB mouth epidermal	Manosroi *et al*., 2006
Thailand	*Ocimum basilicum L.*	P388 murine leukemia	Manosroi *et al*., 2006
Middle East	*Ficus carica*	DJ-75 Burkitts, Jurkat T-cells	Rubnov *et al*., 2001
Jordan	*Allium sativum*	HL60 promyeloleukemic	Li *et al*., 2002
Middle East, Europe	*Silybum marianum*	U87MG glioma	Ramasamy and Agarwal, 2008
Germany	*Viscum album*	COLO colorectal	Khil et al., 2007
South America	*Uncaria tomentosa*	MCF-7 breast	De Martino *et al*., 2006
Brazil	*Lantana fucata*	B16 mouse melanoma, HL-60 leukemia, MCF-7, HCT-8 colon	Dos Santos Júnior *et al*., 2010
Native America	*Phytolacca americana*	B and T cell leukemias	Uckun *et al*., 1986; Waurzyniak *et al*., 1997
		HCT-116	Maness *et al*., 2012
Namibia	*Dicoma capensis*	MCF-7, MCF-12A breast	Steenkamp and Gouws, 2006
South Africa	*Solanum aculeastrum*	HeLa cervical	Koduru *et al*., 2007b
Africa	*Salvia radula*	MCF-7, SF-268 glioblastoma	Kamatou *et al*., 2008

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_{50} values ranging from 4.0-9.4 µM (Chiang *et al*., 2005). In other work, 6-*O*-acyl-*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 prostrate 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* (Sfaxi *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 promyeloleukemic, and HeLa cervical cancer cells (Li *et al*., 2002). IC_{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-Basler *et al*., 2007). 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 (Khil *et al*., 2007).

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

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), IC_{50} 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).
Salvia 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 IC_{50} 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 IC_{50} 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 *Cyathula uncinulata* also had relatively low IC_{50} values of 24.4 µg/ml, 24.8 µg/ml, and 30.0 µg/ml, respectively. The low IC_{50} 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 (Yeager *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; Khambata-Ford *et al*., 2007; Poincloux *et al*., 2009; Nevins, 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 (Khambata-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.

REFERENCES

Aburjai T., Hudaib M., Tayyem R., Yousef M., Qishawi M Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. J Ethnopharmacol. 2007; 110: 294-304.

Allen-Hall L., Arnason J., Cano P., Lafrenie R. *Uncaria tomentosa* acts as a potent TNF-α inhibitor through NF-κB. J Ethnopharmacol. 2010; 127: 685-693.

Amos M., Wu J., Broderick P., Gorlov I., Gu J., Eisen T., Dong Q., Zhang Q., Gu X., Vijayakrishnan J., Sullivan K., Matakidou K., Wang Y., Mills G., Doheny K., Tsai Y., Chen W., Shete S., Spitz M., Houlston R. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nat Genet. 2008; 40: 616-622.

Arayne M., Sultana N., Bahadur S. The berberis story: *Berberis vulgaris* in therapeutics. Pak J Pharm Sci. 2007; 20: 83-92.

Bisi-Johnson M., Obi C., Hattori R., Oshima Y., Li S., Kambizi L., Eloff J., Vasaikar S. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. BMC Complem Altern Med. 2011; 11: 14.

Ceol C., Pellman D., Zon. L. APC and colon cancer: two hits for one. Nat Med. 2007;13: 1286-1287.

Chan P., Tomlinson B. Antioxidant effects of Chinese traditional medicine: focus on trilinolein isolated from the Chinese herb sanchi (*Panax pseudoginseng*). J Clin Pharmacol. 2000; 40: 457-461.

Chang Y., Seo E., Gyllenhaal S., Block K. *Panax ginseng*: a role in cancer therapy? Integr Cancer Ther. 2003; 2: 13-33.

Chen S., Gao J., Halicka H., Huang X., Traganos F., Darzynkiewicz Z. The cytostatic and cytotoxic effects of oridonin (Rubescenin), a diterpenoid from *Rabdosia rubescens*, on tumor cells of different lineage. Int J Oncol. 2005; 26: 579-588.

Chiang Y., Chang J., Kuo C., Chang C., Kuo Y. Cytotoxic triterpenes from the aerial roots of *Ficus microcarpa*. Phytochem. 2005; 66: 495-501.

Clark G., Dressler L., Owens M., Pounds G., Oldaker T., McGuire W. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. New Engl J Med. 1989; 320: 627-633.

De Martino L., Martinot J., Franceschelli S., Leone A., Pizza C. Feo V. De. Proapoptotic effect of *Uncaria tomentosa* extracts. J Ethnopharmacol. 2006; 107: 91-94.

DiPaola R., Zhang H., Lambert Z., Meeker R., Licitra E., Rafi M., Zhu B., Spaulding H., Goodin S., Toledano M., Hait W., Gallo M. Clinical and biologic activity of an estrogenic herbal combination (PC-SPES) in prostate cancer. New Engl J Med. 1998; 339: 85-791.

Dos Santos Júnior H., Oliveira D., de Carvalho D., Pinto J., Campos V., Maurao A., Pessoa C., de Moraes M.,. Costa-Lotufo L. Evaluation of native and exotic Brazilian plants for anticancer activity. J Nat Med. 2010; 64: 231-238.

Ellis C. In keeping with tradition. Nat Rev Drug Discov. 2005 4: 15.

Fukuda K., Hibiya Y., Mutoh M., Koshiji M., Akao S., Fujiwara H. Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells. J Ethnopharmacol. 1999; 66: 227-233.

Gulliksen A., Solli L., Drese K., Sorensen O., Karlsen F., Rogne H., Hovig E., Sirevag R. Parallel nanoliter detection of cancer markers using polymer microchips. Lab Chip. 2005; 5: 416-420.

Halicka H., Ardelt B., Juan G., Mittelman A., Chen S., Traganos T., Darzynkiewicz Z. Apoptosis and cell effects induced by extracts of the Chinese herbal preparation PC-SPES. Int J Oncol. 1997; 11: 437-448.

Holetz F., Pessini G., Sanches N., Cortez D., Nakamura C., Filho B. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem I Oswaldo Cruz. 2002; 97: 1027-1031.

Howe L., Subbaramaiah K., Brown A., Dannenberg A. Cyclooxygenase-2: a target for the prevention and treatment of breast cancer. Endocr-Relat Cancer. 2001; 8: 97-114.

Hunziker-Basler N., Eggenschwiler J., Rist L., Simões-Wüst A., Viviani A. Prolonged cytotoxic effect of aqueous extracts from dried *Viscum album* on bladder cancer cells. Pharmazie. 2007; 62: 237-238.

Jones E. Cancer: Its Causes, Symptoms, and Treatment. Healing Mountain Publishing, Washington (2006).

Kamatou G., Van Zyl R., Davids H., Van Heerden F., Lourens A., Viljoen A. Antimalarial and anticancer activities of selected South African Salvia species and isolated compounds from *S. radula*. S Afr J Bot. 2008; 74: 238-243.

Kemper K. Cat's claw (*Uncaria tomentosa*). The Center for Holistic Pediatric Education and Research. The Longwood Herbal Task Force (1999).

Khambata-Ford S., Garrett C., Meropol N., Basik M., Harbison C., Wu S., Wong T., Huang X., Takimoto C., Godwin A., Tan B., Krishnamurthi S., Burris H., Poplin E., Hidalgo M., Baselga J., Clark E., Mauro D. Expression of epiregulin and amphiregulin and K-RAS mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol. 2007; 25: 3230-3237.

Khil L., Kim W., Lyu S., Park W., Yoon J., Jun H. Mechanisms involved in Korean mistletoe lectin-induced apoptosis of cancer cells. World J Gastroentero. 2007; 13: 2811-2818.

Kienle G., Kiene H. Review Article: Influence of *Viscum album* L (European Mistletoe) Extracts on Quality of Life in Cancer Patients: A Systematic Review of Controlled Clinical Studies. Integr Cancer Ther. 2010; 9: 142-157.

Kim K., Choi C., Kim T., Kwon C., Woo J., Kim Y. Silibinin inhibits glioma cell proliferation via $Ca2^{+}$/ROS/MAPK-dependent

mechanism in vitro and glioma tumor growth in vivo. Neurochem Res. 2009; 34: 1479-1490.

Kitagawa I. Licorice root. A natural sweetener and an important ingredient in Chinese medicine. Pure Appl Chem. 2002; 74: 1189-1198.

Koduru S. Ethnobotanical information of medicinal plants used for the treatment of cancer in the Eastern Cape Province, South Africa Curr Sci. 2007; 92: 906-908.

Koduru S., van de Venter M., Afolayan A. Anticancer activity of steroid alkaloids isolated from *Solanum aculeastrum*. Pharm Biol. 2007; 45: 613-618.

Lamm D., Riggs D. The potential application of *Allium sativum* (garlic) for the treatment of bladder cancer. Urol Clin N Am. 2000; 27: 157-162.

Lampronti I., Martello D., Bianchi N., Borgatti M., Lambertini E., Piva R., Jabbar S., Choudhuri M., Khan M., Gambari R. In vitro antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. Phytomed. 2003; 10: 300-308.

Lansky E., Paavilainen H., Pawlus A., Newman R. *Ficus* spp. (fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. J. Ethnopharmacol. 2008; 119: 195-213.

Lee S., Li M., Tse Y., Leung S., Lee M., Tsui S., Fung K., Lee C., Wayne M. *Paeoniae radix*, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway. Life Sci. 2002; 71: 2267-2277.

Li M., Ciu J., Ye Y., Min J., Zhang L., Wang K., Gares M., Cros J., Wright M., Leung-Tack J. Antitumor activity of Z-ajoene, a natural compound purified from garlic: antimitotic and microtubule-interaction properties. Carcinogenesis. 2002; 23: 573-579.

Lichti-Kaiser K., Staudinger J. The traditional Chinese herbal remedy tian xian activates pregnane X receptor and induces CYP3A gene expression in hepatocytes. Drug Metab Dispos. 2008; 36: 1538-1545.

Lin J., Dong H., Oppenheim J., Howard O. Effects of *Astragali radix* on the growth of different cancer cell lines. World J Gastroenterol. 2003; 9: 670-673.

Maness L., Goktepe I., Hardy B., Yu J., Ahmedna M. Antiproliferative and apoptotic effects of *Phytolacca americana* extracts and their fractions on breast and colon cancer cells. Res J Med Plants. 2012; 6: 17-26.

Manosroi J., Dhumtanom P., Manosroi A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett. 2006; 235: 114-120.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65: 55-63.

Nagrath S., Sequist L., Maheswaran S., Bell D., Irimia D., Ulkus L., Smith M., Kwak E., Digumarthy S., Muzikansky A., Ryan P., Balis U., Tompkins R., Haber D., Toner M. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007; 450: 1235-1239.

Nevins J. The RB/E2F pathway and cancer. Hum Mol Genet. 2001; 10: 699-703.

Nutton V. Ancient Medicine: Sciences of Antiquity. Routledge Publishers, London, England (2004) 123-127.

Poincloux L., Durando X, Seitz J., Thivat E., Bardou V., Giovanni M., Parriaux D., Barriere D., Giovannini M., Delpero J., Monges G. Loss of Bcl-2 expression in colon cancer: A prognostic factor for recurrence in stage II colon cancer. Surg Oncol. 2009; 18: 357-365.

Poller D., Baxter K., Shepherd N. p53 and Rb1 protein expression: are they prognostically useful in colorectal cancer? Brit J Cancer. 1997; 75: 87-93.

Ramasamy K.,. Agarwal R. Multitargeted therapy of cancer by silymarin. Cancer Lett. 2008; 269: 352-362.

Reid B.J., Levine D.S., Longton G., Blount P.L., Rabinovitch P.S. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. Am. J. Gastroenterol. 2000; 95: 1669-1676.

Riva L., Coradini D., Di Fronzo G., De Feo V., De Tommasi N., De Simone F. Pizza C. The antiproliferative effects of *Uncaria tomentosa* extracts and fractions on the growth of breast cancer cell line. Anticancer Res. 2001; 21: 2457-2461.

Ross S. Milk thistle (*Silybum marianum*): An ancient botanical medicine for modern times. Holistic Nursing Practice. 2008; 22: 299-300

Rubnov S., Kashman Y., Rabinowitz R., Schlesinger M., Mechoulam R. Suppressors of cancer cell proliferation from fig (*Ficus carica*) resin: isolation and structure elucidation. J Nat Prod. 2001; 64: 993-996.

Ruiz-Narvaez E., Rosenberg L., Rotimi C., Cupples L., Boggs D., Adeyemo A., Cozier Y., Adams-Campbell L., Palmer J. Genetic variants on chromosome 5p12 are associated with risk of breast cancer in African American women: the Black Women's Health Study. Breast Cancer Res Tr. 2010; 123: 525-530.

Sabová L., Pilátová M., Szilagyi M., Sabo R., Mojzis R. Cytotoxic effect of mistletoe (*Viscum album* L.) extract on jurkat cells and its interaction with doxorubicin. Phytother. Res. 2010; 24: 365-368.

Samowitz W., Slattery M., Sweeney C., Herrick J., Wolff R., Albertsen H. APC mutations and other genetic and epigenetic changes in colon cancer. Mol. Cancer Res. 2007; 5: 165-170.

Sfaxi I., Ferraro D., Fasano E., Pani G., Limam F., Marzouki M. Inhibitory effects of a manganese superoxide dismutase isolated from garlic (*Allium sativum* L.) on in vitro tumoral cell growth. Biotechnol Progr. 2009; 25: 257-264.

Sheng H., Shao J., Kirkland S., Isakson P., Coffey R., Morrow J., Beauchamp R., DuBois R. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. J. Clin. Invest. 1997; 99: 2254-2259.

Sheu Y., Chiang L., Ih-Sheng C., Yu-Chang C., Ian-Lih T. Cytotoxic flavonoids and new chromenes from *Ficus formosana* f. formosana. Planta Med. 2005; 71: 1165-1167.

Shoemaker M., Hamilton B., Dairkee S., Cohen I., Campbell M. In vitro anticancer activity of twelve Chinese medicinal herbs. Phytother. Res. 2005; 19: 649-651.

Silverman K., Koratkar R., Siracusa L., Buchberg A. Identification of the modifier of MIN 2 (MOM2) locus, a new mutation that influences APC-Induced intestinal neoplasia. Genome Res. 2002; 12: 88-97.

Sonoda M., Nishiyama T., Matsukawa Y., Moriyasu M. Cytotoxic activities of flavonoids from two Scutellaria plants in Chinese medicine. J Ethnopharmacol. 2004; 91: 65-68.

Stacey, S., A. Manolescu, P. Sulem, T. Rafnar, J. Gudmundsson, S. Gudjonsson, G. Masson, M. Jakobsdottir, S. Thorlacius, A. Helgason, K. Aben, L. Strobbe, M. Albers-Akkers, D. Swinkels, B. Henderson, L. Kolonel, L. Marchand, E. Millastre, R. Andres, J. Godino, M. Garcia-Prats, E. Polo, A. Tres, M. Mouy, J. Saemundsdottir, V. Backman, L. Gudmundsson, K. Kristjansson, J. Bergthorsson, J. Kostic, M. Frigge, F. Geller, D. Gudbjartsson, H. Sigurdsson, T. Jonsdottir, J. Hrafnkelsson, J. Johannsson,T. Sveinsson, G. Myrdal, H. Grimsson, T. Jonsson, S. Von Holst, B. Werelius, S. Margolin, A. Lindblom A., Mayordomo J., Haiman C., Kiemeney L., Johannsson O., Gulcherhttp J., Thorsteinsdottir U., Kong A., Stefansson K. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nat. Genet. 2007; 39: 865-869.

Steenkamp V., Gouws M. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. S Afr J Bot. 2006; 72: 630-633.

Tillib S., Mirzabekov A. Advances in the analysis of DNA sequence variations using oligonucleotide microchip technology. Curr Opin Biotech. 2001; 12: 53-58.

Tomlinson I., Webb E., Carvajal-Carmona L., Broderick P., Kemp Z., Spain S., Penegar S., Chandler I, Gorman M., Wood W., Barclay E., Lubbe S., Martin L., Sellick G., Jaeger E., Hubner R., Wildhttp R., Rowan A., Fielding S., Howarth K., the CORGI Consortium, Silver A., Atkin W., Muir K., Logan R., Kerr D., Johnstone E., Sieber O., Gray R., Thomas H., Peto J., Cazier Houlston R. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nat Genet. 2007; 39: 984-988.

Uckun F., Gajl-Peczalska K., Kersey J., Houston L., Vallera D. Use of a novel colony assay to evaluate the cytotoxicity of an immunotoxin containing pokeweed antiviral protein against blast

progenitor cells freshly obtained from patients with common B-lineage acute lymphoblastic leukemia. J Exp Med. 1986; 163: 347-368.

Vieira R., Simon J. Chemical characterization of basil (*Ocimum* Spp.) found in the markets and used in traditional medicine in Brazil. Econ Bot. 2000; 54: 207-216.

Wang W., Zhao Y., Rayburn E., Hill D., Wang H., Zhang R. In vitro anti-cancer activity and structure–activity relationships of natural products isolated from fruits of *Panax ginseng*. Cancer Chemot Pharm. 2007; 59: 589-601.

Watt JM and Breyer-Brandwijk MG. The medicinal and poisonous plants of southern and eastern Africa. Edinburgh & London: E. & S. Lívingstone Ltd (1962) 958-963.

Waurzyniak B., Schneider E., Tumer N., Yanishevski Y., Gunther R., Chelstrom L., Wendorf H., Myers D., Irvin J., Messinger Y., Ek O., Zeren T., Langlie M., Evans W., Uckun F. In vivo toxicity, pharmacokinetics, and antileukemic activity of TXU (anti-CD7)-pokeweed antiviral protein immunotoxin. Clin Cancer Res. 1997; 3: 881-890.

Xie J., Wang C., Wicks S., Yin J., Kong J.,. Li J., Li Y., Yuan C. *Ganoderma lucidum* extract inhibits proliferation of SW 480 human colorectal cancer cells. Exp Oncol. 2006; 28: 25-29.

Yeager M., Orr N., Hayes R., Jacobs K., Kraft P., Wacholder S., Minichiello M., Fearnhead P., Yu K., Chatterjee N., Wang Z., Welch R., Staats B., Calle E., Feigelson H., Thun M., Rodriguez C., Albanes D., Virtamo J., Weinstein S., Schumaker F., Giovannucci E., Willett W., Cancer-Tassin G., Cussenot O., Valeri A., Andriole G., Gelmann E., Tucker M., Gerhard D., Fraumeni J., Hoover R., Hunter D., Chanock S., Thomas G. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nat Genet. 2007; 39: 645-649.

Zhang, Y., Qian R., Li P. Shikonin, an ingredient of *Lithospermum erythrorhizon*, down-regulates the expression of steroid sulfatase genes in breast cancer cells. Cancer Lett. 2009; 284: 47-54.

Zhu M., Li X., Zhao S. Digital candidate gene approach (DigiCGA) for identification of cancer genes. Method Mol Biol. 2010; 653: 105-129.