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Application of Bioassay in the Safety and/or Quality Control of Herbal Products

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ABSTRACT

Bioassays are useful tools in modern biosciences. The applications include development of new drugs; measurement of biological activity in undefined substances; investigation of endogenous mediator function; determination of drug toxicity; and measurement of the concentration of known substances such as pollutants or herbal products. As medicinal plants are very popular on market, the safety and/or quality control of these phytotherapeutic agents become an important issue. Currently, botanical identification, molecular identification and chemical analysis are often utilized for the quality analysis of plant products. However, the methods are not totally reliable due to factors such as seasonal and geographical variations in plant growth. An important development of herbal product quality analysis has been the use of biological assay systems which can test many samples for activity in a short period of time. The advantages of using bioassays in this aspect are many-fold. The tests can avoid the ethical and financial constraints of using human subjects or larger animals. Bioassay-guided isolation of 'active' compounds can be achieved and much smaller amount of test material is needed. However, it is important to realize that there is no single method to assure absolute quality. Bioassays combined with plant macro and microscopic data, chemical profiling and DNA fingerprinting would probably offer the safest and most efficacious herbal products.

Key words: bioassay, safety, quality control, herbal products, botanical identification, molecular identification, chemical analysis

INTRODUCTION

The term "bioassay" is a common short form for "biological assay". Bioassay can be defined as those tests which are used to detect the biological or functional activity of an extract or pure substance from herbs and/or natural products using the living organism, tissue or cell. Bioassay may be used for qualitative and/or quantitative characterizations. Qualitative bioassays are used for assessing the biological effects of a substance that may not be quantified, such as abnormal development or deformity. Quantitative bioassays involve the estimation of effective concentration or potency of a substance by measurement of the biological response that it produces. Bioassays are essential tools in the development of new drugs. Additionally, important applications of bioassay include: measurement of the biological activity of undefined substances; investigation of the functions of endogenous mediator; determination of the side-effect profile, including the degree of drug toxicity; measurement of the concentration of known substances; and assessing the amount of pollutants being released by a particular source.

QUALITY ANALYSIS OF HERBAL PRODUCTS

Medicinal plants have become very popular as botanical supplements in recent years. Herbal products are gaining an important share on the global pharmaceutical market. In the United States, it was estimated that expenditures for alternative medicine professional services increased 45.2% between 1990 and 1997 and Americans consumed \$5.1 billion dollars worth of herbal medicine in 1997⁽¹⁾. Thus, the quality control of these phytotherapeutic agents is an important issue. Reliable authentication procedures for plant materials are critical for the protection of both the public and industry. Several methods are commonly employed for the quality analysis of herbal products. Non-quantitative methods include:

I. Botanical control using histological identification: Macroscopic and microscopic examinations can be used as rapid and inexpensive identification techniques⁽²⁾.

II. Quality identification by chemical analysis: Measurement of the principal ingredient of the herbal extract is a commonly used quantitative method. Chemical analysis is by far the best method for the detection of contam-

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inants and can be an excellent method for plant identification⁽²⁾. For example, in addition to microscopic examination, one could use fluorescent or UV spectrophotometer, thin-layer chromatography (TLC), Capillary electrophoresis (CE), or high-performance liquid chromatography (HPLC) to identify and determine the quality of *Smilax glabra Roxb* (Tufuling)^(3,4).

III. Molecular identification: Molecular methods such as those involving DNA sequencing⁽⁵⁾, polymerase chain reactions (PCR)⁽⁶⁾, Amplified Fragment Length Polymorphism (AFLP)⁽⁷⁾, Restriction Fragment Length Polymorphism (RFLP)⁽⁸⁾, Random Amplified Polymorphic DNA (RAPD)⁽⁹⁻¹²⁾, Inter-Simple Sequence Repeat (ISSR)^(13,14), gene probes (or biological chip)⁽¹⁵⁾ have been used for herbal DNA identification^(2,16-18).

In table 1, we show the classical identifications of natural products and list the advantages as well as limitations. There are a number of shortcomings accompanying the use of these methods:

I. Seasonal changes of plant may lead to changes in compositions. For example, the chemical components of essential oil from *Baeckea frutescens* collected from different seasons have some different characteristics⁽¹⁹⁾.

II. Different growing years of plants have different bioactivities. Dai *et al.* used thermokinetic parameters of *E. Coli* metabolism to identify the quality of Rhizoma Coptid in different growing years⁽²⁰⁾.

III. Regional variations in plant constituents can exist. It has been demonstrated that there are significant differences in yield and quality of *Scutellaria baicalensis* from different habitats. The crude drug from Pingyi (Shandong province) is better than those from other habitats⁽²¹⁾.

IV. The principal ingredient may not be the most biological active component.

V. The methods used in harvesting and storage affect the herbal compositions.

VI. Different manufacturing processes lead to products with different biological effectiveness.

BIOASSAY FOR HERBAL PRODUCTS

An important development of drug discovery process over the last several decades has been the use of biological assay systems which can test many samples for activity in a short period of time, thus providing enough data for biostatistical analysis. Although clinical trials involving human subjects could be considered as bioassays in the broadest sense, these are generally excluded from

Table 1. Classical identification of herbal products

Methods	Classical identification of herbal products	
	Histological identification	Molecular identification
Macroscopic and microscopic identification	Spectrophotometry, TLC, CE, and HPLC	DNA sequencing, PCR, AFLP, RFLP, RAPD, ISSR, Biological chip
Example	Identify <i>Retinervus Citri Reticulatae</i> and its confused species by FTIR ⁽²⁹⁾ Twenty-two Shayuanzi samples collected from all over the country were identified by TLC ⁽³⁰⁾ Characterization chemical compounds in Jin-Guo-Lan (<i>Tinospora sagittata</i> and <i>Tinospora capillipes</i>) by HPLC ⁽³¹⁾	Identify ginseng by DNA-based protocol ⁽⁵⁾ Identify niuzi from different area by RAPD ⁽¹²⁾ Biological chip coupled with PCR technique was developed for rapid authentication of ginseng species ⁽¹⁵⁾
Advantages	Best way for contaminants detection and plant identification	Identify different species
Limitations	Only detects chemicals that are present in plants Misleading the Seasonal, environmental, individual variation among plants or bastards	Secondary metabolites can interfere or damage DNA

this category. Depending on the methodology involved, bioassays may be divided into “*in vivo*”, “*ex vivo*” or “*in vitro*”. “*Ex vivo*” means living animal tissue is used; “*in vivo*” means living animals are used. “*In vitro*” test is used to investigate the effect of compounds or extracts which do not involve either living animal tissue or whole animals. *In vitro* tests commonly utilize cells, enzymes or isolated receptors as targets for the substances under test, but in some cases, small whole animals can be used, e.g. the use of the nematode *Caenorhabditis elegans* for anthelmintic studies⁽²²⁾.

The advantages of using bioassays for quality analysis of herbal products are many-fold:

I. The tests can avoid the ethical and financial constraints of using human subjects or larger animals.

II. Bioassay-guided isolation of ‘active’ compounds can be achieved.

III. Much smaller amount of test material is needed.

In the early days of development about three decades ago, bioassays were used to study the extracts and/or compounds for antibacterial and antifungal activity. These were followed by cytotoxicity tests on mammalian cells for discovering novel anticancer agents⁽²³⁾. More recently, bioassays have been utilized in the quest for antiviral agents including potential compounds for treating HIV⁽²⁴⁾. Although *in vivo* models give a more accu-

rate reflection of the activity of substances used in traditional medicine, their use in many countries is severely restricted due to economic and ethical concerns, and this has resulted in the widespread use of *in vitro* tests in ethnopharmacological studies. Such tests are very useful where the identity of compounds responsible for the biological activity of an extract is being investigated and where limited supplies of material are available. For instance, in the development of agents used to treat type-2 diabetes or NIDDM, a method of measuring insulin release from pancreatic cells has been introduced whereby radioimmunoassay is used to measure insulin levels after treating cultured islet cells with the test substances. Thirty-two plant species for treating diabetes were put to this test and three showed a stimulatory effect⁽²⁵⁾. An ethanolic extract of *Gymnema sylvestre*, a species well known for its antidiabetic properties, showed a significant increase in insulin secretion from three different cultured islet beta-cell lines⁽²⁶⁾. In table 2, we further conclude some *in vitro* and *in vivo* bioassays models of natural products and give the specific example.

On the other hand, it is important to realize that for any particular disease, multiple pathological factors may be involved. The use of only one bioassay gives a very incomplete picture of the effect of the extract on the disease as a whole. A symptom may be due to a number of disease states and, consequently, a variety of mechanisms may serve as targets for bioassays. In a similar way, it is very unusual for there to be only one target for a particu-

Table 2. Bioassay applied in quality and safety control of herbal products

Assays/ Measurements/ Models	Examples of application
<i>In vitro</i>	
Reporter genes assay	One could screen the bioactivity of natural products by DNA manipulation and promoter system. For example, curcumin is discovered as an inducer of the heat shock response by reporter assay ^(32,33) .
Advanced glycation end products (AGEs) production	AGEs is related to diabetic complications. Jang <i>et al</i> measured the AGE production and discovered Puerariafuran is an inhibitor of AGEs ⁽³⁴⁾ .
Phospholipid liposome peroxidation	Inhibition of phospholipid liposome peroxidation is a marker of antioxidant. Aruoma <i>et al</i> used this marker to identify the antioxidant activity of <i>Rosemary</i> ⁽³⁵⁾ .
Anti-endotoxin effect	LPS induced cytokine gene, such as TNF α or IL-1, expression patterns are anti-endotoxin markers. Detection of those changes of patterns would discover anti-endotoxin components, <i>Isatis indigotica</i> or <i>Rhizoma Chuanziong</i> , for example, in herbal products ⁽³⁶⁾ .
Cytotoxicity of cancer cell lines	The anti-cancer bioactivity of plant derived compounds could be tested by the IC50 of various cancer cell lines ⁽³⁷⁾ .
<i>In vivo</i>	
Hepatoprotection/ CCl4 induced liver damage	Carbon tetrachloride (CCl4) is used as a hepatotoxicant. Protein isolate of <i>P. niruri</i> protects liver tissues against oxidative damage ⁽³⁸⁾ .
Diabetes/ STZ or fructose induced diabetic rats	Ginsenoside Rh2 is demonstrated as an ideal anti-diabetic medicine by either STZ-induced type I diabetic or fructose-induced type II diabetic animal ^(39,40) .
Hyperlipidemia/ High-fat diet induced hyperlipidemia hamsters	Hypolipidemic effects of <i>Monascus</i> and <i>Tamarindus indica</i> L. were tested on hyperlipidemic hamsters ^(41,42) .
Angiogenesis/ Chick embryo chorioallantoic membrane (CAM) model	CAM is an classical angiogenesis model ⁽⁴³⁾ . <i>Epimedium sagittatum</i> , <i>Trichosanthes kirilowii</i> and <i>Dalbergia odorifera</i> showed the strong angiogenetic activity in CAM model ⁽⁴⁴⁾ .

lar disease so a variety of test systems must be employed. Therefore, batteries of test systems used to test plants and other materials for potential usefulness in wound-healing, diabetes, cancer and dementia have been developed⁽²⁷⁾.

CONCLUSIONS

Authentication of herbal products is an important issue for the protection of consumers. Safety and/or quality control must be exerted throughout the various processing stages, from the raw material to the finished product. Bioassays have brought about a useful approach to the quality analysis and development of herbal products for medicinal use. It can be anticipated that such applications will continue to grow. However, it is very important to realize the strength and limitation of these tests. For example, one should not extrapolate results from *in vitro* tests to claim clinical efficacy without taking into account other biopharmaceutical factors. Development of bioassay systems with batteries of tests targeting a particular disease will overcome the current shortcomings of using a single test. As for authentication of herbal products, currently there is no single method to assure absolute quality. Bioassays combined with plant macro and microscopic data, chemical profiling and DNA fingerprinting would probably offer the safest and most efficacious herbal products.

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REFERENCES

- Eisenberg, D. M., Davis, R. B., Ettner, S. L., Appel, S., Wilkey, S., Van Rompay, M. and Kessler, R. C. 1998. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA* 280: 1569-1575.
- Techen, N., Crockett, S. L., Khan, I. A. and Scheffler, B. E. 2004. Authentication of medicinal plants using molecular biology techniques to compliment conventional methods. *Curr. Med. Chem.* 11: 1391-1401.
- Ng, T. B. and Yu, Y. L. 2001. Isolation of a novel heterodimeric agglutinin from rhizomes of *Smilax glabra*, the Chinese medicinal material tufulin. *Int. J. Biochem. Cell Biol.* 33: 269-277.
- Xia, F., Yu, C. L. and Wang, Y. T. 2006. The development of quality control and pharmacological research on *Smilax glabra* Roxb. *Zhong Yao Cai* 29: 516-519.
- Mihalov, J. J., Marderosian, A. D. and Pierce, J. C. 2000. DNA identification of commercial ginseng samples. *J. Agric. Food Chem.* 48: 3744-3752.
- Pan, H., Huang, F., Wang, P., Zhou, L., Cao, L. and Liang, R. 2001. Identification of *Amomum villosum*, *Amomum villosum* var. *xanthioides* and *Amomum longiligulare* on ITS-1 sequence. *Zhong Yao Cai* 24: 481-483.
- Ha, W. Y., Shaw, P. C., Liu, J., Yau, F. C. and Wang, J. 2002. Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). *J. Agric. Food Chem.* 50: 1871-1875.
- Wang, C. Z., Li, P., Ding, J. Y., Fishbein, A. and Yuan, C. S. 2007. Discrimination of *Lonicera japonica* THUNB. from different geographical origins using restriction fragment length polymorphism analysis. *Biol. Pharm. Bull.* 30: 779-782.
- Hon, C. C., Chow, Y. C., Zeng, F. Y. and Leung, F. C. 2003. Genetic authentication of ginseng and other traditional Chinese medicine. *Acta Pharmacol. Sin.* 24: 841-846.
- Lin, H. and Lin, J. 2003. RAPD analysis of four species of *Cuscuta* in Shandong Province. *Zhong Yao Cai* 26: 8-10.
- Wang, P., Huang, F., Zhou, L., Cao, L., Liang, S., Xu, H. and Liu, J. 2000. Analysis of *Amomum villosum* species and some adulterants of zingiberaceae by RAPD. *Zhong Yao Cai* 23: 71-74.
- Zheng, X. Z., Guo, B. L. and Yan, Y. N. 2002. Study on the genetic relationship between Chinese and Korean medicinal materials of niuxi with the method of RAPD. *Zhongguo Zhong Yao Za Zhi* 27: 421-423.
- Gui, F. R., Guo, J. Y. and Wan, F. H. 2007. Genetic variations among populations of *Ageratina adenophora* from different areas, investigated by inter-simple sequence repeat markers. *Fen Zi Xi Bao Sheng Wu Xue Bao* 40: 41-48.
- Hu, Y., Zhang, Q., Xin, H., Qin, L. P., Lu, B. R., Rahman, K. and Zheng, H. 2007. Association between chemical and genetic variation of *Vitex rotundifolia* populations from different locations in China: its implication for quality control of medicinal plants. *Biomed. Chromatogr.* 21: 967-975.
- Qin, J., Leung, F. C., Fung, Y., Zhu, D. and Lin, B. 2005. Rapid authentication of ginseng species using microchip electrophoresis with laser-induced fluorescence detection. *Anal. Bioanal. Chem.* 381: 812-819.
- Lee, J. S., Lim, M. O., Cho, K. Y., Cho, J. H., Chang, S. Y. and Nam, D. H. 2006. Identification of medicinal mushroom species based on nuclear large subunit rDNA sequences. *J. Microbiol.* 44: 29-34.
- Yip, P. Y. and Kwan, H. S. 2006. Molecular identification of *Astragalus membranaceus* at the species and locality levels. *J. Ethnopharmacol.* 106: 222-229.
- Zhang, T., Xu, L. S., Wang, Z. T., Zhou, K. Y., Zhang,

- N. and Shi, Y. F. 2005. Molecular identification of medicinal plants: *Dendrobium chrysanthum*, *Dendrobium fimbriatum* and their morphologically allied species by PCR-RFLP analyses. *Yao Xue Xue Bao* 40: 728-733.
19. Liu, B. M., Lai, M. X., Liang, K. N., Chen, Y., Yan, K. J., Cai, Q. L. and Li, Y. H. 2004. Study on quality analysis of essential oil from twig and leaf of *Baeckea frutescens*. *Zhongguo Zhong Yao Za Zhi* 29: 539-542.
 20. Dai, C. M., Xiao, X. H., Wang, D., Sun Y. Q., Jin, C., Fan, D. L., Zhao, Y. L., Liu, Y. and Zhao, X. J. 2006. Quality evaluation of Rhizoma Coptidid in different growing years by microcalorimetry. *Chin. Tradit. Herbal Drugs* 37: 205-209.
 21. Yu, J., Chen, J., Xiao, X. Y., Zhu, X. H., Yang, S. L. and Cheng, H. Z. 2005. Study on yield and quality of *Scutellaria baicalensis* from different habitats. *Zhongguo Zhong Yao Za Zhi* 30: 491-494.
 22. Perrett, S. and Whitfield, P. J. 1995. Atanine (3-dimethylallyl-4-methoxy-2-quinolone), an alkaloid with anthelmintic activity from the Chinese medicinal plant, *Evodia rutaecarpa*. *Planta. Med.* 61: 276-278.
 23. Suffness, M. 1987. *Biologically Active Natural Products*. p.283. Clarendon Press. Oxford, U. K.
 24. Cardellina, J. H., Munro, M. H. G. and Fuller R. W. 1993. A chemical screening strategy for the dereplication and prioritisation of HIV-inhibitory aqueous natural products extracts. *J. Natur. Products* 56: 1123-1129.
 25. Srijayanta, P. M. J., S. Persaud, M. J. and Lawrence, A. Raman 1998. *In vitro* screening of medicinal plants for potential antidiabetic effects. *J. Pharm. Pharmacol.* 50: 219.
 26. Persaud, S. J., Al-Majed, H., Raman, A. and Jones, P. M. 1999. *Gymnema sylvestre* stimulates insulin release *in vitro* by increased membrane permeability. *J. Endocrinol.* 163: 207-212.
 27. Houghton, P. J., Howes, M. J., Lee, C. C. and Steventon, G. 2007. Uses and abuses of *in vitro* tests in ethnopharmacology: visualizing an elephant. *J. Ethnopharmacol.* 110: 391-400.
 28. Wang, S. Y., Zhang, M. and Wang, Z. T. 2004. Microscopic identification of homonym drugs of "Baitou-weng" by digital imaging technique. *Yao Xue Xue Bao* 39: 797-802.
 29. Sun, Y., Cheng, C., Xu, M. and Tu, Z. 2002. Direct identification of *Retinervus Citri Reticulatae* from its confused species by FTIR. *Zhong Yao Cai* 25: 783-786.
 30. Zhang, Y. J., Zhang, J. J., Yan, X. L., Zhang, R., Tang, Y. and Liu, Y. 2003. Identification and evaluation of TCM shayuanzi. *Zhongguo Zhong Yao Za Zhi* 28: 496-499.
 31. Zhang, Y., Shi, Q., Shi, P., Zhang, W. and Cheng, Y. 2006. Characterization of isoquinoline alkaloids, diterpenoids and steroids in the Chinese herb Jin-Guo-Lan (*Tinospora sagittata* and *Tinospora capillipes*) by high-performance liquid chromatography/electrospray ionization with multistage mass spectrometry. *Rapid Commun. Mass Spectrom.* 20: 2328-2342.
 32. Dunsmore, K. E., Chen, P. G. and Wong, H. R. 2001. Curcumin, a medicinal herbal compound capable of inducing the heat shock response. *Crit. Care Med.* 29: 2199-2204.
 33. New, D. C., Miller-Martini, D. M. and Wong, Y. H. 2003. Reporter gene assays and their applications to bioassays of natural products. *Phytother. Res.* 17: 439-448.
 34. Jang, D. S., Kim, J. M., Lee, Y. M., Kim, Y. S., Kim, J. H. and Kim, J. S. 2006. Puerariafuran, a new inhibitor of advanced glycation end products (AGEs) isolated from the roots of *Pueraria lobata*. *Chem. Pharm. Bull. (Tokyo)* 54: 1315-1317.
 35. Aruoma, O. I., Spencer, J. P., Rossi, R., Aeschbach, R., Khan, A., Mahmood, N., Munoz, A., Murcia, A., Butler, J. and Halliwell, B. 1996. An evaluation of the antioxidant and antiviral action of extracts of rosemary and Provençal herbs. *Food Chem. Toxicol.* 34: 449-456.
 36. Liu, Y. H., Liu, Y. F. and Guo, X. X. 2001. Current studies on anti-endotoxic chemical components of traditional Chinese medicine in China. *Acta Pharmacol. Sin.* 22: 1071-1077.
 37. Kim, J. and Park, E. J. 2002. Cytotoxic anticancer candidates from natural resources. *Curr. Med. Chem. Anticancer Agents* 2: 485-537.
 38. Bhattacharjee, R. and Sil, P. C. 2007. Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties. *Food Chem. Toxicol.* 45: 817-826.
 39. Lai, D. M., Tu, Y. K., Liu, I. M., Chen, P. F. and Cheng, J. T. 2006. Mediation of beta-endorphin by ginsenoside Rh2 to lower plasma glucose in streptozotocin-induced diabetic rats. *Planta. Med.* 72: 9-13.
 40. Lee, W. K., Kao, S. T., Liu, I. M. and Cheng, J. T. 2007. Ginsenoside Rh2 is one of the active principles of *Panax ginseng* root to improve insulin sensitivity in fructose-rich chow-fed rats. *Horm. Metab. Res.* 39: 347-354.
 41. Lee, C. L., Tsai, T. Y., Wang, J. J. and Pan, T. M. 2006. *In vivo* hypolipidemic effects and safety of low dosage *Monascus* powder in a hamster model of hyperlipidemia. *Appl. Microbiol. Biotechnol.* 70: 533-540.
 42. Martinello, F., Soares, S. M., Franco, J. J., Santos, A. C., Sugohara, A., Garcia, S. B., Curti, C. and Uyemura, S. A. 2006. Hypolipemic and antioxidant activities from *Tamarindus indica* L. pulp fruit extract in hypercholesterolemic hamsters. *Food Chem. Toxicol.* 44: 810-818.
 43. Norrby, K. 2006. *In vivo* models of angiogenesis. *J. Cell Mol. Med.* 10: 588-612.
 44. Wang, S., Zheng, Z., Weng, Y., Yu, Y., Zhang, D., Fan, W., Dai, R. and Hu, Z. 2004. Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. *Life Sci.* 74: 2467-2478.