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Voucher Specimens are Essential for Documenting Source Material Used in Medicinal Plant Investigations

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Abstract

Plant-based natural products research is conducted using a wide variety of source material. The plant material is often obtained directly from the wild, from cultivated plants originally obtained from the wild, or purchased in raw or processed forms. In plant science a voucher specimen usually consists of a pressed, dried herbarium specimen with detailed collection data and serves as a record of an individual plant in time and space. This review article describes why vouchering is necessary and documents actual examples of how improper vouchering can result in serious problems. The primary reason for vouchering is to have a permanent record documenting the material that was used in a particular study. If a specimen is not saved or is not made available to others, the true identity of the plant materials used in a research project may be questioned. Due to the morphological and chemical complexities of interspecific hybrids, within-species variation, and the difficulty associated with identifying species in certain plant genera, the preservation of vouchers is essential for the documentation of the identity and source of such plant material. The use of best practices in specimen preparation is critical for successful documentation. The lack of proper voucher specimens for some research projects has led to serious problems, such as the inability to reproduce critical results, the association of chemical data with the wrong genus and species, and even the complete rejection of the published research results. In cases where plant material was initially misidentified and properly prepared voucher specimens were available, the identities of the research material were eventually corrected and the data was subsequently associated with the correct species, retaining the inherent scientific value of the research.

Introduction

The Earth is home to a great diversity of plant species with estimates of valid, described species currently ranging from 200,000 to 420,000 in number (Nic Lughadha et al., 2005). This extensive diversity of plants with the associated phytochemical variation, is a primary reason why humans have been able to discover and utilize myriads of plant-based natural products. Some of earliest scientific works of literature were herbals and materia medica devoted to documenting plant species of medical and economic value used by the early Egyptian, Sumerian, Indian,

Chinese, and Greek civilizations (Evans, 2002). Unfortunately, the interpretation of these descriptions and/or primitive illustrations for correct modern plant identification can be quite difficult (Buenz et al., 2004; Riddle, 1996). Collections of medicinal plants were cultivated in gardens, and the earliest herbaria (reference collections of dried, pressed plant specimens often mounted on paper or in books) were composed of plant specimens being grown in physic (medicinal plant) gardens. An example is the Oxford University Herbarium, the oldest herbarium in the United Kingdom and the fourth oldest such collection in the world. This herbarium was established in 1621 to document the plants growing in the Oxford Physic Garden (Oxford University Herbaria, 2011). Since those early days of botanical history, many herbaria have grown in both the scope of their collections and in total number of specimens.

In addition to overall species diversity, significant variation can occur at the within-species (intraspecific) level. A herbarium may contain numerous specimens of a single species that were collected from different localities and/or collected on different dates and in some cases spanning great distances both spatially (from different continents) and temporally (from different centuries). The sum total of all the collections of that species, held in many herbaria around the world, serve as the best scientific record (however incomplete) of the morphological and anatomical variation as well as distribution of that particular species. Herbaria managers strive to provide optimal conditions to ensure long-term preservation of botanical specimens. This includes using acid-free paper and glue for the mounting of specimens, as well as storage in protective cases and environments that will reduce the risk of damage caused by insects, heat, high humidity, and infrastructure issues (sprinkler-type fire suppression systems, leaky roofs and pipes, and other structural failures).

In plant science, a voucher specimen usually consists of a pressed, dried herbarium specimen with detailed collection data. A voucher serves as a permanent record and reference of an individual plant in time and space. This voucher record documents the existence of the plant material, and in the case of research studies, the plant that was used in a study. Properly prepared voucher specimens, must have the necessary plant parts (usually vegetative material including roots if possible, and flowers and/or fruits) to enable reliable plant identification. The voucher should be housed in a collection that is accessible to other researchers in perpetuity. If a properly prepared voucher is available, the most basic foundation of the research, the plant material that was actually used, can be verified and the conclusions of the research can be confidently associated with that species. If a specimen is not saved or is not made available to others, the true identity of the plant materials used in a research project may be questioned.

The importance of a voucher sample for research on plants is illustrated by contrasting of examples of research projects that properly vouchered plant material against research conducted with unvouchered or improperly vouchered plant material. The examples of inadequate vouchering document cases where source materials were either improperly identified and where the identifications could not be confirmed. The lack of proper voucher specimens in these examples led to serious problems such as the inability to reproduce critical results, the association of chemical data with the wrong genus and species, and even the complete rejection of the published research results. The complexities of properly identifying interspecific hybrids and an example of distinct intraspecific variation are discussed to further enforce the need for proper documentation of plant material used in research endeavors. Other examples describe how initial misidentifications of research material were eventually corrected as a result of having properly prepared voucher specimens and an example of a properly vouchered bioexploration project is presented.

Why Voucher?

Reproducibility is critical to conducting successful scientific research. For example, when phytochemical and biological assays are conducted mistakes in species identification of the sample material are possible, even with the assistance of botanical specialists. This misidentification of plants can be particularly true when screening species from notoriously difficult to identify genera (*Artemisia*,

Astragalus, *Crataegus*, *Mentha*, *Origanum*, *Rubus*, and *Salix* for temperate zone examples), plants collected from regions of high biodiversity, and plants collected without the necessary components for proper identification (usually flowers or fruits). In addition, species delimitation and taxonomy can change with additional taxonomic research, so vouchers provide a method to update species identifications as new plant classifications are accepted. If at all possible, a voucher should be prepared from the same individual plant that provided the sample used for the biological or chemical analysis, and both plant fractions should be collected at the same time to reduce potential collecting errors. If individual plants are too small for both a bulk sample and a voucher, a representative from the same population is necessary.

For numerous species of plants, within-species (intraspecific) variation has been documented for characteristics, such as anatomy, morphology, phytochemical content, and DNA sequences (Cordell et al., 1998; Koornneef et al., 2004; Lila, 2006; Manners and Davis, 1984). A complication with phytochemical data is that in many instances constituent content of plants can vary significantly within populations and individual plants as a result of phenological stage, time of day or year, and in response to environmental factors, such as altitude, nutrient stress, and herbivory (Karlova, 2006; Kennedy and Barbour, 1992; McDougal and Parks, 1984; Stevens and Lindroth, 2005; Witzell et al., 2003). This intraspecific and intraindividual variation can have a significant impact when a particular plant species is being evaluated for a potentially beneficial biological activity, since variation might be closely related to the bioactivity being sought.

Commercially purchased raw material from wholesale purveyors can pose serious problems associated with vouchering. Commercial material is frequently not associated with a preserved herbarium specimen, and species identification may not have been conducted by trained taxonomists. In addition, many herbaria lack the facilities to properly voucher dried commercial botanicals (powdered or whole). In some instances, bulk material can be adequately identified to species by morphological, chemical, or molecular analysis (Joshi and Khan, 2006; World Health Organization, 1998; Zerega et al., 2002), and in such cases, a bulk reference voucher sample can be prepared from the raw material. Such reference samples should be labeled with the species name, the commercial source, batch number, and date of receipt. If possible, precise locality data should be obtained from the purveyor and placed on the label. Protocols for the proper preparation of commercially obtained bulk materials have been described in detail by Hildreth et al. (2007). The ideal solution would be if bulk providers could provide a properly prepared voucher specimen with an order of their plant materials. If they are unable to do so, there is a possibility that the origin and species accuracy of their material are questionable.

Studies conducted using commercially available plant preparations have additional complications associated with vouchering. The confirmation of the botanical components contained within or used to prepare these preparations relies solely on the manufacturer. Variation between commercial products makes adequately documenting the company, specific product, and the batch/production number necessary (Draves and Walker, 2003; Gurley et al., 2000; Monmaney, 1998). In addition, samples of the packaged material should be saved for future reference.

A review of the materials and methods presented in 81 different MEDLINE-indexed, randomized, controlled trials evaluating single-herb preparations of echinacea, garlic, ginkgo, saw palmetto, and St. John's wort published between 2000 and 2004, Wolsko et al. (2005) found that characterization of herbal supplements was often lacking. The few studies that did quantify some of the chemical constituents reported variation from the expected content (Wolsko et al., 2005). In some cases, significant standardization of commercial botanical extracts occurs. One such example is the *Ginkgo biloba* L. extract EGb 761, a standardized extract of ginkgo leaves that contains approximately 24% flavone glycosides (primarily quercetin, kaempferol and isorhamnetin) and 6% terpene lactones (2.8-3.4% ginkgolides A, B and C, and 2.6-3.2% bilobalide) (Anonymous, 2003). With this type of standardization, problems of misidentification are less likely, but not all commercial preparations have such rigorous production standards

and in some cases can contain adulterants and contaminants (Gilroy et al., 2003; Slifman et al., 1998). To verify the chemical content of botanical products and to allow for better comparison of results between studies, the utilization chromatographic fingerprints and quantitative analyses are beneficial as documentation in bioactivity based research (Miller and Applequist, 2006).

In studies on aromatherapy, documenting the chemical make-up of the volatile oil is particularly important. For example, commercial rosemary oil is extremely variable in spite of the attempts of ISO (International Organization for Standardization) at standardization. Commercial rosemary (*Rosmarinus officinalis* L.) oils may be high in 1,8-cineole, borneol, bornyl acetate, camphor, α-pinene, β-thujone, myrcene, verbenone, 1-octen-3-ol, or any of the other principal constituents. If a "rosemary" oil is beneficial for a specific physiological or psychological response, but the oil is not characterized, then research connected with the oil may not be reproducible. Likewise, many aromatherapy studies with "lavender oil" (*Lavandula angustifolia* Mill.) appear, on closer examination, to have been done with lavandin oil (*Lavandula x intermedia* Emeric ex Loisel.), which contains a lower percentage level of linalool/linalyl acetate then lavender oil. With no characterization, however, no way of knowing which oil was used is available and thus, makes the research irreproducible.

The major benefit of having a properly prepared voucher is that the specimen can be reexamined at a later time. Permanent and public repositories in herbaria (often at universities, botanical gardens, and museums) should be used for storing voucher specimens. These herbaria allow the public access to their collections and/or their information. Index Herbariorum (IH), a global database of public herbaria, can be consulted to locate contact information for such herbaria (Thiers, continuously updated). Since the reason for preparing a specimen as a voucher is to enable availability for others to examine and verify the plant material, vouchers that reside in a local research laboratory or commercial facility are less likely to be available to the public, and if improperly stored (such as in a lab bench drawer), may eventually be at risk of being discarded or destroyed.

Re-examination of plant material after obtaining strange activity results may reveal that the plant being studied was originally misidentified. Collaboration between natural product researchers and botanists are the most effective way of ensuring that plants being studied are identified correctly. A herbarium voucher or a highly resolved digital photograph of the herbarium sheet can easily be sent to an expert for identification or annotation. To this effect, Nation Center for Complementary and Alternative Medicine of the National Institutes of Health (NIH) has included in the Natural Product Integrity policy (NCCAM, 2010) the following statement:

> "Investigators must demonstrate that their investigative team has the appropriate product and analytical expertise to select the test and placebo agents for study and to ensure the product integrity. For example, botanists trained in taxonomy may be required to identify voucher specimens accurately."

Lack of vouchered specimen

A number of issues associated with vouchering have led to the incorrect identification of a species, resulting in chemical analyses and/or biological activities being associated with the wrong species. German researchers, conducting phytochemical analyses on unvouchered plant material imported from the U.S. and assumed to be the roots of *Echinacea purpurea* (L.) Moench. (Asteraceae), reported the isolation and structural determination of the first non-volatile sesquiterpene constituents to be found in the genus *Echinacea* (Bauer et al., 1985). These investigators also reported that these isolated compounds exhibited bioactivity in immunological activity tests and were probably contributing to the immunostimulating activity of *E. purpurea*. Because the report was published as a preliminary communication, detailed materials and methods were not included, but were to be published at a later date. Only later was the studied plant material determined be *Parthenium integrifolium* L. and not *Echinacea purpurea* (Bauer et al., 1987). Commercially available plant material purported to be *E. purpurea* has sometimes been adulterated with *Parthenium integrifolium*, as well as a number of other species (Kindscher, 1989).

If a crude extract of a species is found to have a

positive result in a biological assay, additional plant collections will likely be necessary to conduct further analyses, such as bioassay guided fractionation. The sourcing of additional material may be critical to additional screening and further study, to the reproducibility of the assay results, and for further discovery of bioactive components. For testing, additional plant material should be from the same wild source as the original voucher material to ensure genotypic similarity. If the new material is not obtained from the same individual as the original sample, the new material should be vouchered as well.

Although collaboration between botanists and chemists is extremely important for the initial collection and identification of plant materials, a need also exists for documentation at later stages of research, such preparation for publications. Botanists collaborating with chemists need to inform and educate colleagues about proper vouchering methods and providing detailed voucher information in all publications. Making vouchers is extremely important, but sharing the information about where the vouchers are stored is also critical. Voucher information is being increasingly required by many journals publishing articles on plant-based chemistry research.

Ietswaart (1980), in his revision of the genus *Origanum* (Lamiaceae), stated:

> "None of the chemical data mentioned have been used as criteria for delimitation of *Origanum*, its sections or species. The first reason for this is that the data are too fragmentary. Secondly, many authors gave incomplete or inaccurate data about morphology, geography and taxonomy of the plants…,"

that is, none of the chemical reports could be verified by vouchers, and all were essentially useless in a taxonomic context. Vouchering is not restricted to documenting plant-based studies. In a paper on amatoxins and phallotoxins in *Amanita* mushroom species, Yocum and Simons (1977) addressed the issue of proper identification in mycological studies:

> "Many reports on chemical analysis of mushrooms do not include sufficient data to defend the identification of the species analyzed. Such omission is unfortunate, because mushroom taxonomy is far from being a precise, routine science. At best, other investigators are deprived of information they would like to have, and at worst they can be misled by very accurate analyses on very wrong material."

Fungi are also saved in herbaria. Mycological collections usually consist of fungal dried specimens kept in index card sized paper or waxed-paper packets, with a label affixed to the outside, detailing the collection data for the specimen.

Funk et al., (2005) described the unfortunate situation that occurred because of a nearly ubiquitous practice of not vouchering plant material used in chromosome counts conducted prior to 1965. Although the data generated during that time period comprises a significant portion of the cytological record, many researchers will not utilize data from those reports because the identity of the plants cannot be verified. Variation in ploidy level could be attributed to natural variation or could be based on misidentifications, but no way of determining this exists without a voucher.

Vouchers make a difference

If a voucher specimen has been properly prepared and a later evaluation of that specimen leads to a redetermined identity, all the associated research data still remains valuable and can be associated with the new species identification. An important example concerning species identification occurred when a bulk sample with a sterile voucher was collected in Cameroon as part of an initiative of the National Cancer Institute to search for novel anti-HIV compounds from natural sources. The collected plant material, originally identified as *Ancistrocladus abbreviatus* Airy Shaw (Ancistrocladaceae), exhibited anti-HIV activity with positive results from two novel bioactive alkaloids (michellamines) isolated via bioassay guided fractionations (Manfredi et al., 1991). In publiccation of the findings, the general locality of the collection site was described and the collector was thanked in the acknowledgements, but no reference to the existing voucher specimen was provided. Subsequent experimentation required more plant material and an additional bulk sample of *A. abbreviatus* was obtained from a different locality in Gabon. Upon follow-up chemical and bioactivity testing, no evidence of michellamines could be determined in the new plant material, and this new plant material exhibited no activity against HIV (Boyd et al., 1994). Yet, because an adequate voucher specimen with

sufficient locality data had been collected, the original population was eventually revisited and additional plant material with flowers and fruits were collected and studied. The population was re-identified as a new plant species, *Ancistrocladus korupensis* D.W. Thomas & Gereau (Thomas and Gereau, 1993). Upon analysis of additional plant material collected from this specific population, both michellamines and anti-HIV activity were once again detected (Boyd et al., 1994). Yet, the publication reaffirming anit-HIV activity only described the general locality of the collection site, but again made no reference of a voucher specimen. *A. korupensis* has subsequently been the subject of many chemical analyses and numerous novel biological active compounds with either anti-malarial or HIV-inhibiting activity being discovered, yet voucher specimens are referenced in only a few of these studies (Hallock et al., 1994; Hallock et al., 1995; Hallock et al., 1997; McCloud et al., 1997; McMahon, et al., 1995).

In the above example, the original voucher for the plant material used by Manfredi et al. (1991) lacked floral parts and was misidentified, but the existence of a voucher with detailed geographic data allowed for the recollection of material from the original collection site. Having adequate locality information for a collection is a crucial element of a properly prepared specimen. But this example also shows that collection of specimens with adequate parts for identification is critical. Of course, a sterile voucher is better than no voucher, and sometimes plant species can be identified based on vegetative characteristics. But, if at all possible, sterile vouchers should be avoided.

In additional to medicinal species, vouchers serve the same critical purpose with other plants. In an extensive career studying the genetics of sex determination in the Amaranthaceae, M.J. Murray conducted numerous experimental hybridizations resulting in literally thousands of progeny (Murray, 1940). To be certain of the identification of the parental species being used in his crosses, Murray sent specimens to one of the foremost experts on Amaranthaceae, P.C. Standley of the Field Museum in Chicago. In addition to having an expert identify his material, Murray also made sure to prepare voucher specimens of his parental species as well as some of his resulting hybrids. Later, another researcher working on Amaranthaceae taxonomy and genetics, J.D. Sauer, examined a few of the parent species used by Murray and determined that they were incorrectly identified. The fact that these specimens existed enabled Sauer to re-identify the specimens and to explain some of the more unusual results Murray had reported (Sauer, 1953). Sauer stated that the main purpose of his article was not to discuss the "specific factual details of Murray's findings, but to call attention to his method of procedure as a case study in identification of research material." Sauer went on to explain that by making voucher specimens:

> "…[Murray] effectively safeguarded results based on years of work with over 50,000 plants. Any qualified person who questions the identity of this material has only to send for the specimens in order to see for himself exactly what Murray worked with and what he meant by each name he used. Thus any taxonomic changes required by increasing knowledge of the group become no more than minor details. There is no possibility in this case that an otherwise competent investigation will become meaningless simply because the identity of the research material cannot be established."

This above example verifies that vouchers can and do serve the function of providing a permanent record. Even if the voucher sample is misidentified, the plant can be annotated with a new species name and previous research based on that material can be reinterpreted and still remain informative, as opposed to misinforming those who reference the material or the research results becoming useless.

Murray also documented his genetic work in *Mentha* (Lamiaceae; Tucker and Kitto, in press), preparing thousands of herbarium vouchers from 1954-1986, that are now deposited at the Delaware State University Claude E. Phillips Herbarium (DOV). These specimens allow an expanded interpretation of his work, as he confused *M. canadensis* L. with *M. arvensis* L. and *M. suaveolens* Ehrh. with *M.* x *rotundifolia* L., along with confusion over other species due to the inadequate cataloging and describing the plants of a region in floras of that time. Properly filed vouchers also allowed the reinterpretation of molecular studies in the Lamiaceae in which

M. suaveolens was misidentified as *M. rotundifolia* from inadequate floras (Kaufmann and Wink, 1994; Prather et al., 2002).

The complexities of intraspecific variation

The species *Artemisia dracunculus* L. (tarragon; Asteraceae) is a widespread, morphologically diverse, herbaceous perennial plant (Hall and Clements, 1923) with a long history of human use. The uniquely fragrant variety French tarragon (*A. dracunculus* var. *sativa* Besser) is used as a culinary herb. Wild or Russian tarragon (*A. dracunculus*, numerous varieties) has been utilized as a medicinal herb throughout its native range (western North America, Asia and Eastern Europe) for the treatment of a variety of ailments (Khalmatov et al., 1984; Moerman, 2003; Uphof, 1968). Like many other species in the genus *Artemisia*, *A. dracunculus* produces a wide array of useful phytochemicals including alkaloids, flavonoids, monoterpenoids, sesquiterpenoids, coumarins, isocoumarins, and polyacetylenes (Aglarova et al., 2008).

In addition to the most common ploidy state as a diploid species, *A. dracunculus* is known to have an extensive series of polyploidy cytotypes within the same species (Eisenman and Struwe, 2011). In a study of polyacetylenes in the plant roots, Greger (1979) observed that the cytotypes of *A. dracunculus* (diploid, hexaploid, octoploid and decaploid) had distinct qualitative differences in their phytochemistry. A study from the late 1980s, on the effect of tarragon on streptozotocin-induced diabetes in mice showed that herbal extracts of this species reduced hyperphagia and polydipsia (Swanston-Flatt et al., 1989). Accordingly to the study, the plant material used for the experiment was purchased from a retail herbalist in Birmingham, U.K., but no further information about the source was presented and no voucher specimen was cited. This species has more recently been the subject of numerous additional diabetes-related studies, and through a bioassay-guided fractionation a number of the specific bioactive compounds have been identified (Govorko et al., 2007; Logendra et al., 2006; Ribnicky et al., 2006; Schmidt et al. 2007; Wang et al., 2008).

With the goal of assessing chemical variation of the anti-diabetic compounds in different germplasm material, a study was conducted using *A. dracunculus* from a wide variety of sources, including wild collected material from the U.S. and Kyrgyzstan and purchased commercial seed of wild and French tarragon (Eisenman, 2010). The experimental results clearly showed that phytochemical content was highly dependent on the source of the material, and that qualitative phytochemical variation was correlated with the ploidy level of the plants (Table 1) (Eisenman, 2010). This level of complexity in phytochemical content is another example of vouchering being necessary for a study to be reproducible. The data showed only some cytotypes of *A. dracunculus* contained the bioactive compounds of interest, and the vouchers provided evidence that the plants were properly identified and observed chemical variation was not the result of analyzing some other *Artemisia* species mistakenly identified as *A. dracunculus*. Similar situations of intraspecific chemical variation have been documented in other medicinal plants, such as *Echinacea spp*., Kava (*Piper methysticum* G. Forst.), and North American Ginseng (*Panax quinquefolius* L.; Assinewe et al., 2003; Binns et al., 2002; Lebot et al., 1999). With this level of chemical complexity the utilization of chromatographic fingerprints as documentation in bioactivity based research may be necessary (Miller and Applequist, 2006).

Table 1. Intraspecific variation of medicinally active compounds in cytotypes of *A. dracunculus*.

Bioactive compound	2*n**	4*n*	8*n*	10*n*
Davidigenin	–	–	–	+
2,4-dihydroxy-4-methoxydihydrochalcone	–	–	–	+
Sakuranetin	+	–	–	+
6-demethoxycapillarisin	+	–	–	+

*2*n* = diploid, 4*n* = tetraploid, 8 *n*= octaploid, 10*n* = decaploid; The presence (+) and absence (-) of the compound is indicated. Data adapted from Eisenman et al. (2011).

Vouchered bioinvestigations

The International Cooperative Biodiversity Group (ICBG) Program is a U.S. government-funded program devoted to a collection-based exploration of bioactive small molecules, proteins, and metabolic pathways derived from biological organisms worldwide. The goal of the program was to identify poten-

tial lead candidates for medical drugs, crop protection, and bioenergy development. The Central Asia ICBG program ran from 2003-2008 and was led by research teams from Rutgers University and the University of Illinois Champaign-Urbana, along with Central Asian collaborators. The project focused on screening plants, endophytic fungi and soil inhabiting bacteria from Kyrgyzstan and Uzbekistan for biological activity against a number of human diseases.

Because of the wide-scale sampling of species (over 1600) conducted for the ICBG program, a manual containing standardized methods for the collection and processing of both bulk samples and associated vouchers were developed to assure accuracy (icbg.rutgers.edu/datacollection.htm). In association with this manual, detailed field collection forms were prepared to streamline the documentation process. The use of the data collection forms ensured that all samples had the proper data associated with the voucher specimen and bulk material regardless of the team conducting the field collection.

During processing of voucher collections for the ICBG Central Asia, a specimen labeled as *Sorbus tianschanica* (Rosaceae) was obviously not a *Sorbus* species, but was in fact a species of *Crataegus* (Rosaceae). If no voucher had been available, the extract prepared from the bulk material would have been associated with the wrong species and any chemical and bioactivity data would have been incorrectly associated with *Sorbus tianschanica*. By having a properly prepared voucher, this misidentification was easily corrected.

Discussion

Voucher specimens provide a permanent, physical record and form the foundation on which all natural product research stands. In all cases, two or more duplicate voucher specimens should be prepared and one of these can easily be sent to a taxonomic expert anywhere in the world for confirmation of the species identification. Detailed information on procedures to properly collect, press, and prepare voucher specimens are available (Hildreth et al., 2007). The preparation of additional voucher specimens is advisable, and can serve back-ups in case damage to or loss of the main voucher specimen.

In making vouchers of plant material being studied or marketed, the specimens must be prepared properly or any scientific or identity assurance value is lost. Misidentification can occur as a result of mislabeling, especially where labels are printed en masse for a set of specimens. Such labels are sometimes hastily added to unmounted voucher specimens in folded newspapers or collection bags, leading to the wrong label being placed on the wrong specimen.

Buying commercial or collecting wild seed is a common practice in many laboratories and businesses. Some researchers and growers assume that the species being used is that listed on the package label. Verifying the identification of all material grown from seed is essential and a voucher specimen should be prepared indicating the company providing the seed and if possible, the provenance of the seed. In a worst case scenario, seed could be purchased, grown, and the identification never verified. For the researcher, this could mean years of studies and multiple publications associated with the wrong species or possibly even the wrong genus. For the grower and processor, this could mean entire productions of plants and products being removed from market shelves. Vouchers should be prepared and the identity of the plant material confirmed before publication of research or sale of the plant material.

Vouchers help deal with changes in plant taxonomy and changes in the environment. The taxonomy of plants is not static and revisions in plant classification occur. New data can result in an updated understanding of species and subspecies within a genus. Species can be split into two species or subspecies and a voucher can be critical in determining which of these taxa were actually used in a particular chemical or bioactivity study. Botanists often make initial identifications in the field during the collection of specimens and having a properly prepared voucher allows the initial identification to be confirmed at a later time when appropriate resources (microscopes, floras, identification manuals) are available. Cultivated plants are generally less well represented in herbaria, but a definite need to document these plants, particularly those used in research and commercial enterprises exists.

The examples from Murray's complex hybrids (presented above) demonstrate the importance of vouchers for cultivated material. Although a living plant in an arboretum or botanical garden may be sampled for a study, the accession (the sampled living plant) will eventually die and therefore cannot serve as a permanent voucher. A properly prepared and stored voucher of the plant, however, would always be available. Even wild plant populations can change over time, and revisiting a population at a later date does not mean the exact same living organism will be present. A voucher documents a plant found at a specific place at a specific time.

The U.S. Food and Drug Administration is pro-voucher. The Guidance for Industry: Botanical Drug Products (FDA, 2004) states:

> "A suitable voucher specimen (reference specimen) for each of the botanical raw materials should be established, along with a reference standard for the drug substance and drug product."

This simple practice should be the first step at the beginning of any research and commercial endeavor using plant materials for chemical or bioactivity analyses. For example, the McCormick Science Institute (MSI; a research-oriented organization of McCormick & Company, Inc.) has followed FDA recommendations by obtaining botanical identifications and depositing vouchers of their dried botanicals, at the Claude E. Phillips Herbarium.

Herbarium specimens can and have been used to investigate phytochemical variation within previously collected plants. Zangerl and Berenbaum (2005) studied changes in toxic furanocoumarins in specimens of the invasive weed *Pastinaca sativa* L. (Apiaceae) by analyzing herbarium specimens collected over a period of 152 years. This time period represented the before and after introduction to North America the major herbivore of the plant species, *Depressaria pastinacella* (Duponchel, 1838), commonly known as parsnip webworm. The preservation of phytochemicals in herbarium specimens is highly dependent on the type of chemical compound, the drying process used to prepare the specimen, and the environmental conditions of the herbarium where the voucher is housed.

The issue of vouchering has been the subject of numerous commentaries about vouchering in research of plant, fungal, and animal taxonomy and in systematics and ecology (Anonymous, 2000; Ammirati, 1979; Funk et al., 2005; Goldblatt et al., 1992; Ruedas et al., 2000). In a paper on amatoxins and phallotoxins in *Amanita* mushroom species, Yocum and Simons (1977) addressed the issue of proper identification in mycological studies with:

> "Many reports on chemical analysis of mushrooms do not include sufficient data to defend the identification of the species analyzed. Such omission is unfortunate, because mushroom taxonomy is far from being a precise, routine science. At best, other investigators are deprived of information they would like to have, and at worst they can be misled by very accurate analyses on very wrong material."

To describe and publish a new plant taxon (species, subspecies, variety, cultivar, or selection), the International Code of Botanical Nomenclature (ICBN) requires in Article 37.7 that a voucher specimen (designated as a holotype) be prepared or designated from previously collected herbarium material, and that the herbarium in which the type is conserved be specified (ICBN, 2006). This voucher serves the same purpose as all other vouchers, to provide a permanent record of material the taxonomic author was studying and the information used to describe the new species. This preservation enables others to see the actual specimen used to define the new taxon. In Article 7A.1 by ICBN states:

> "It is strongly recommended that the material on which the name of a taxon is based, especially the holotype, be deposited in a public herbarium or other public collection with a policy of giving bona fide researchers access to deposited material, and that it be scrupulously conserved."

If these requirements are not met, the new taxon will not be accepted by the botanical community.

The preparation and proper storage of vouchers can also include provide plant tissue for DNA analysis. Using PCR based methods such AFLPs, SNPs and microsatellites, the potential to identify particular genotypes associated with characters such as high chemical yield and reduced toxicity exist. Genetic fingerprinting methods have the potential for use in

species identification, the detection and characterization of contaminants, and, possibly, the identification of the geographical origin of a sample (Smillie and Khan, 2010). Most professional journals now require that DNA and amino acid sequences intended for publication be submitted to a sequence database, such as GenBank before being published, but many journals do not yet require that vouchers be made for the plants from which these sequences were isolated (Pleijel et al., 2008). While GenBank serves as an archival database to which submitters are responsible for providing the taxonomic identification for their entries, submission of a voucher and voucher information is only encouraged for submission with sequence data. Any requirements for vouchering lie with individual journals (Federhen et al., 2009). Funk et al., (2005) discussed the importance of vouchering for molecular studies stating:

> "…some researchers collect all of their own experimental material, but most get at least some samples from herbaria, botanical gardens, or other collectors, often as a leaf or two sent in silica gel or even as extracted DNA. Few systematists could tell if the plant sent to them is a species of *Oenothera* or *Camissonia*, or for that matter *Arabidopsis*, if all they receive is a few leaves or extracted DNA. Even when the investigator personally takes material from an herbarium sheet, the identification may or may not be correct….Without vouchers, the enormously costly and time-consuming extractions, sequencing, alignments, and analyses may be worthless, since there can be no serious questioning or reexamination of results and conclusions."

When compared with the complexities of modern chemical analysis and studies on pharmacological activity, plant identification and preparing a voucher specimen may seem to be the most basic of scientific endeavors. Yet, without conducting this fundamental practice, researchers have the risk of having their work invalidated (Flaster and Lassiter, 2004; Funk et al., 2005). In these times of mass throughput screening and genomics, researchers are capable of producing vast amounts of data, making the ability to organize, manage, and archive this data increasingly important. Similar to Ammirati (1979), our intention is not to criticize researchers who unknowingly neglected to taxonomically document their work, but rather to raise awareness regarding the extreme importance of preparing voucher collections. Without adequately prepared vouchers, a study cannot be confirmed or disconfirmed. Thus, the question remains: Are you 100% sure of the identity of the plant material that you are grinding and extracting? If not, why bother doing the research?

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