Pharmaceutical Crops: An Overview

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Abstract: *Pharmaceutical crops* is an ambiguous term used by biologists and chemists for different categories of plants. This review focuses on the definition and scope of pharmaceutical crops. We define pharmaceutical crops as those cultivated species that are used for extraction or preparation of therapeutic substances such as active pharmaceutical ingredients (APIs), excipients used in pharmaceutical formulations, vaccines and antibodies, as well as other therapeutic proteins. Based on the type of pharmaceutical product, these crops can be classified into three distinct yet sometimes overlapping categories: crops for the production of small therapeutic molecules (STMs), large therapeutic molecules (LTMs), or standardized therapeutic extracts (STEs). This review briefly discusses the relationships of pharmaceutical crops with traditional food crops, medicinal plants, medicinal crops, and invasive species. It also addresses the importance, advantages, problems, and challenges of research and development of pharmaceutical crops.

Keywords: Pharmaceutical crops, definition, active pharmaceutical ingredients, small therapeutic molecules, large therapeutic molecules, standardized therapeutic extracts, medicinal plants, medicinal crops.

WHAT ARE PHARMACEUTICAL CROPS?

Pharmaceutical crops is an ambiguous term used by scientists of varying disciplines referring to different categories of plants and their utilization. Biologists often define *pharmaceutical crops* as genetically modified (GM) or engineered crops to produce vaccines, antibodies, and other therapeutic proteins [1-4], but sometimes, other terms for such a class of crops or practice are used. For example, *pharma crops* is used to designate transgenic plants for the production of pharmaceuticals (e.g., antibiotics, diagnostic compounds, antibodies, vaccines, etc.) or industrially-useful biomolecules (e.g., biodegradable plastics, engine oils, food processing enzymes, etc.), rather than for the production of food, feed or textile fibers [1]. *Biopharming* means a practice of using GM or engineered crops (e.g., tobacco, maize, soybeans, tomato, rice, wheat, potato, safflower, alfalfa, and leaf mustard) as bioreactors to produce large therapeutic molecules [5]. However, natural product chemists occasionally use the term *pharmaceutical crops* for a different class of plants, those that produce pure small molecules as active pharmaceutical ingredients, although there is not any clear definition [6, 7]. These pharmaceutical ingredients are naturally-occurring single entities of secondary metabolites in plants. Well known examples for this chemical definition are *Taxus* spp. (Taxaceae) and *Podophyllum* spp. (Berberidaceae) for production of anti-cancer drugs, and *Artemisia annua* L. (Asteraceae) for an anti-malarial drug. The different meanings of *pharmaceutical crops* as used by biologists and chemists may not only cause confusion in academia and industry, but also may often mislead the public.

We define the term *Pharmaceutical Crops* as those cultivated species that are used for the extraction or preparation of therapeutic substances such as active pharmaceutical ingredients (APIs), excipients used in pharmaceutical formulations, vaccines and antibodies, as well as other therapeutic proteins. Based on the type of pharmaceutical product, these crops can be classified into three distinct yet sometimes overlapping categories: crops for the production of small therapeutic molecules (STMs), large therapeutic molecules (LTMs), and standardized therapeutic extracts (STEs) (Table **1**). Pharmaceutical crops can be either terrestrial or aquatic species. Although marine organisms have shown promising potential in drug discovery [8-10], this review focuses on examples of terrestrial plants as pharmaceutical crops. It is estimated that there are 400-500 plant species currently man-

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Table 1. Summary of Three Types of Pharmaceutical Crops

	Pharmaceutical Crops for the Production of		
	Small Therapeutic Molecules (STMs)	**Large Therapeutic Molecules (LTMs)**	**Standardized Therapeutic Extracts (STEs)**
Therapeutic Substances			
Molecule Type	Basically secondary metabolites	Basically primary metabolites	Both
Molecular Weight	Low molecular weight (usually <1,000)	High molecular weight (usually 10,000 to 100,000)	Usually of low molecular weight
Molecular Origin	Endogenous	Endogenous or exogenous	Endogenous
Purity	Pure	Pure	Mixture
***In vitro* Production**	Possible but most are not commercially feasible yet	Possible but most are not commercially feasible yet	May be unable to produce the same quality products
Biotransformation	Possible and relatively easy	No data	No data
Quality Control	Relatively easy	Relatively easy	Relatively difficult
Crops			
Type	Traditional (possible Transgenic in the future)	Traditional or Transgenic	Traditional
Cultivation History	< 100 yrs	Non-transgenic crops: <100 yrs Transgenic crops: <20 yrs	Many cultivated for centuries
Induction	Possible by stresses	No data	Difficult to manage
Ethnobotanic Uses	Many are used in traditional medicines	Transgenic crops are not used in traditional medicines	Usually used in traditional medicines

aged as pharmaceutical crops for production of STMs, fewer species are used for production of LTMs, and thousands of species are managed as crops for STEs.

Pharmaceutical Crops for the Production of Small Therapeutic Molecules (STMs)

This group of pharmaceutical crops produces STMs (usually having a molecular weight of less than 1,000 Daltons) as either APIs or their precursors. Typically, these small molecules are secondary metabolites. The plants producing these STMs are often managed as new or potential crops *via* intact plant systems, tissue or cell culture systems.

Some crops in this category are well known for the production of promising active ingredients which are used directly or semi-synthetically modified as anti-cancer drugs. In the late 1960s, Dr. Monroe Wall, Dr. Mansukh Wani, and colleagues at the Research Triangle Institute isolated and characterized the anti-tumor pentacyclic alkaloid camptothecin (CPT) (**1**) (Fig. **1**) from the wood and bark of *Camptotheca acuminata* Decne. (Cornaceae) [11]. Because CPT (**1**) is insoluble in water, its water-soluble sodium salt was used in the initial clinical trials of the 1970s, but the results

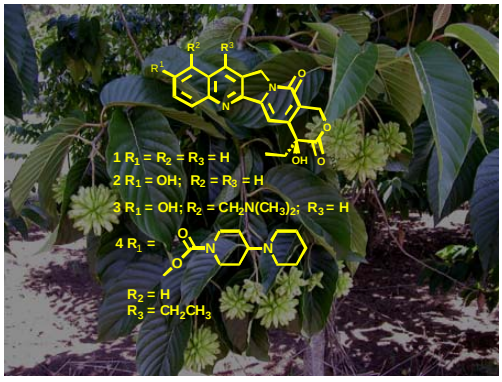

Fig. (1). Camptothecin (**1**) and 10-hydroxycamptothecin (**2**), two natural alkaloids isolated from *Camptotheca* spp. and their semi-synthetic anti-cancer drugs topotecan (**3**) and irinotecan (**4**) (*Camptotheca acuminata* cultivated in Texas, USA, photo by S.Y. Li).

were not promising [12]. Interest in CPT drugs was not rekindled until its unique mechanism of action was discovered. In 1985, Hsiang *et al.* found that CPT traps the enzyme topoisomerase I (TOPI), in complex with DNA, thus preventing cancer cell DNA replication and killing tumor cells [13]. From 1985 to 1995, extensive research efforts were focused on developing water-soluble and bioactive analogs of natural CPT (**1**) and 10-hydroxycamptothecin (HCPT) (**2**) (Fig. **1**). In the mid-1990s, two CPT analogs, topotecan (Hycamtin®) (**3**) (Fig. **1**) and irinotecan (trade names Camptosar® and Campto, also known as CPT-11) (**4**) (Fig. **1**), received the United States Food and Drug Administration (FDA) approvals and have been primarily used in patients with advanced ovarian and metastatic colorectal cancers, respectively. In the last decade, the global annual sales of the two CPT drugs totaled approximately $1 billion. The semi-synthetic production of these two CPT drugs and several other CPT analogs for clinical trials requires CPT (**1**) or HCPT (**2**) as precursors.

Camptotheca species (known by various common names including happytree, tree of life, cancer tree in English and xi shu in Chinese) are still a major source of CPT (**1**) and HCPT (**2**). The genus currently includes three species of deciduous trees (*C. acuminata*, *C. lowreyana* S. Y. Li, and *C. yunnanensis* Dode) and its range is restricted to remote areas in southern China [14]. No wild trees of *C. acuminata* were identified in a 1994-1997 national survey of that nation, although this species is commonly cultivated as an urban street tree in southern China. In 1997, it was listed as an endangered species in China. Currently, fruits or leaves are harvested from cultivated *C. acuminata* trees along roads or small plantations for CPT (**1**) and HCPT (**2**) extraction. *Camptotheca lowreyana* and *C. yunnanensis* have small wild populations with tens of mature trees only in Guangdong and Yunnan provinces. Four high CPT-yielding cultivars of shrubs or trees were developed to harvest leaves and stems for sustainable production of CPTs: *C. lowreyana* 'Katie', 'CT168', and 'Hicksii', and *C. yunnanensis* 'Tropic' [14, 15]. A cultivation technique to enhance biosynthesis of CPTs in *Camptotheca* was developed [16].

Taxus L. (yew) is another example of an important pharmaceutical crop utilized for anti-cancer drug production. In the early 1970s, Wall, Wani, and co-workers isolated and elucidated the structure of the terpene paclitaxel (Taxol®) (**5**) (Fig. **2**) from the bark of Pacific yew (*Taxus brevifolia* Peattie) (Fig. **2**), an evergreen and coniferous tree of the Taxaceae from the old-growth forests of the North American Pacific Northwest [17, 18]. The Pacific yew was long considered a "trash tree" [18]. Susan Horwitz demonstrated that paclitaxel's unique antimitotic mechanism of action is to promote microtubule assembly and inhibit mitosis rather than preventing the formation of microtubules as with previous anti-cancer drugs [19, 20]. The unique structure and mode of action stimulated global interest in the drug's development. In the last two decades, paclitaxel (with various trade names including Taxol®, Onxal™, Onxol®, Abraxane®, Apo-Paclitaxel®, Asotax, Bristaxol, Cryoxet, and Praxel) has been one of the most widely used chemotherapy agents in the world, particularly in patients with advanced and metastatic ovarian and breast cancers. Docetaxel (Taxotere®) (**6**) (Fig. **2**), a semi-synthetic analog is mainly used to treat non-small cell lung cancer. Ortataxel (**7**) (Fig. **2**), a third generation taxane is now in Phase II clinical trials against taxane-resistant breast cancer [21].

The early production of paclitaxel (**5**) relied on the bark of Pacific yew, with a limited supply of this nonrenewable source. The yield of paclitaxel (**5**) from the yew bark is tremendously low; with 3,000 yew trees being needed to harvest enough bark to produce 1 kg of paclitaxel (**5**). Currently, paclitaxel (**5**), docetaxel (**6**), and ortataxel (**7**) can be produced by semi-synthesis using 10-deacetylbaccatin III (10-DAB) (**8**) (Fig. **2**) and other baccatins isolated from needles of European yew (*T. baccata* L.) and other yew species [22, 23]. Over 400 taxanes have been isolated from various species of *Taxus* [24]. Species cultivated for production of paclitaxel (**5**) since the 1990s include *T. brevifolia* in North America, *T. baccata* in Europe, *T. wallichiana* Zucc. (syn. *T. yunnanensis* W. C. Cheng & L. K. Fu), *T. cuspidata* Sieb. & Zucc. in China, and *T. canadensis* Marshall in Canada. In China, for example, 6672 hectares of *Taxus* plantations, including the species *T. madia* (*T. cuspidata* × *T. baccata*), *T. wallichiana, T. wallichiana* var. *chinensis* (Pilger) Florin, and *T. cuspidata,* were located in 19 provinces in May 2005 [25]. Although numerous companies supply *Taxus* seedlings, a high-yielding, fast growing cultivar is not yet available [25].

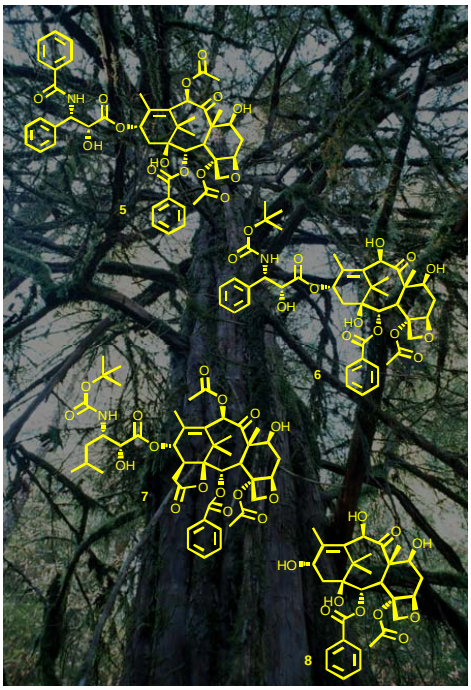

Fig. (2). Anti-cancer taxane drugs paclitaxel (**5**), docetaxel (**6**), and ortataxel (**7**) and their precursor 10-deacetylbaccatin III (**8**) isolated from *Taxus* spp. (*Taxus brevifolia* in the Pacific Northwest, USA: Courtesy of www.stevenfoster.com).

Two other well-known pharmaceutical crops cultivated for anti-cancer drug production are *Podophyllum* L. (mayapple) and *Catharanthus roseus* (L.) G. Don (Madagascar periwinkle or rosy periwinkle). *Podophyllum* is a genus of six species of herbaceous perennial plants native to eastern Asia (five species) and eastern North America (one species). Eastern Asian *P. emodi* Wall. (syn. *P. hexandrum* Royle) and North American *P. peltatum* L. (Fig. **3**) have long been used in traditional medicine. Toxic podophyllin (a resin) from an ethanol extract of the rhizomes has been used to treat warts. In 1880, podophyllotoxin (**9**) (Fig. **3**), an aryltetrainlactone cyclolignan, was isolated from *P. peltatum* rhizomes [24]. Etoposide (**10**) (Fig. **3**) and teniposide (**11**) (Fig. **3**), two semi-synthetic analogs of podophyllotoxin (**9**), are potent DNA TOPII cancer drugs used for small cell lung and testicular cancers and lymphomas/leukemias; likewise the water-soluble etoposide phosphate (also known as etopophos) (**12**) is used for refractory testicular cancer and small cell lung cancer (Fig. **3**). At present, both *P. emodi* and *P. peltatum* are cultivated for isolation of podophyllotoxin (**9**) [26, 27]. *Catharanthus roseus* (also known as *Vinca rosea* L., family Apocynaceae) (Fig. **4**) is an evergreen perennial species native and endemic to Madagascar, but now naturalized throughout the tropics and widely sold as a cultivated plant elsewhere. It is used for production of vinblastine (**13**) and vincristine (**14**) (Fig. **4**), two well-known antimitotic cancer drugs used to treat Hodgkin's lymphoma and acute childhood lymphoblastic leukemia, respectively. Vinorelbine (Navelbine®) (**15**) and vindesine (Eldisine®) (**16**) (Fig. **4**), two synthetic drugs derived from vinca alkaloids, are used to treat non-small cell lung and advanced breast cancers, acute lymphoblastic leukemia, and malignant melanoma [24].

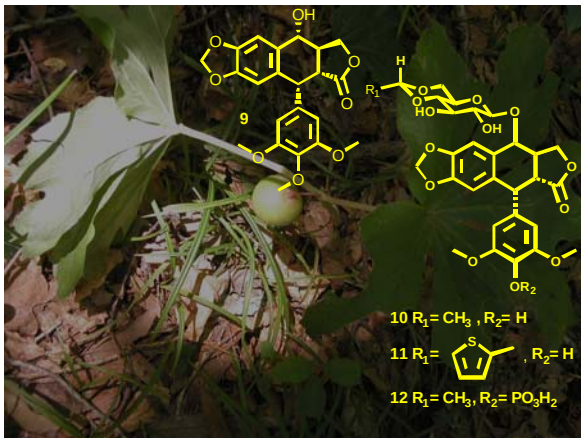

Fig. (3). Podophyllotoxin (**9**), a natural lignan isolated from *Podophyllum* spp., and semi-synthetic anti-cancer drugs etoposide (**10**), teniposide (**11**), and etoposide phosphate (**12**) (*Podophyllum peltatum* in Texas, USA: by S.Y. Li).

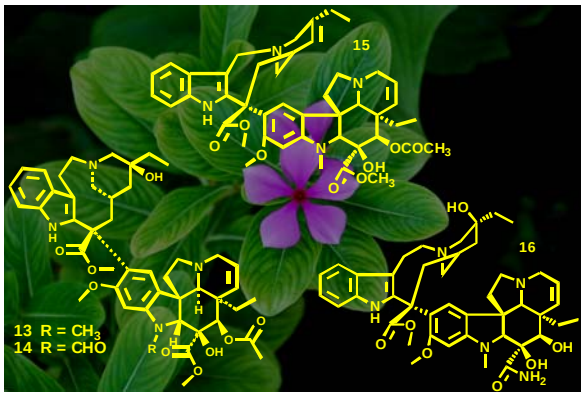

Fig. (4). Vinblastine (**13**) and vincristine (**14**), two natural vinca alkaloids isolated from *Catharanthus roseus*, and semi-synthetic anti-cancer drugs vinorelbine (navelvine) (**15**) and vindesine (eldisine) (**16**) (*Catharanthus roseus,* photo by M.J. Balick).

Active ingredients from several other pharmaceutical crops are currently being evaluated in cancer drug clinical trials, but severe side effects due to toxicity challenge drug development. For example, *Cephalotaxus* Sieb. & Zucc. (Cephaltaxaceae) native to southeastern Asia (including *C. fortunei* Hooker, *C. sinensis* (Rehder & E. H. Wilson) Li, *C. oliveri* Mast., *C. mannii* Hooker, and *C. harringtonia* (Forbes) K. Koch.) are used to isolate the anti-tumor agents harringtonine (**17**) and homoharringtonine (**18**) (Fig. **5**), two alkaloids used as cancer drugs in China [28]. Homoharringtonine (**18**) is being evaluated in clinical trials for treating myeloid leukemia in the United States, but has severe side effects [24]. Similarly, colchicine (**19**) isolated from *Colchicum autumnale* L. (Liliaceae) (Fig. **6**) is used to treat gout and familial Mediterranean fever. The alkaloid and its natural analog thiocolchicine (**20**) (Fig. **6**) demonstrate antileukemic activity by inhibiting the polymerization of tubulin, but both are highly toxic [24].

Some pharmaceutical crops are managed for the production of other categories of drugs. *Artemisia annua* L., commonly known as wormwood or qinghao, is an annual herbaceous species native to China and now cultivated throughout the world for production of the sesquiterpene lactone artemisinin (**21**) (Fig. **7**) [7]. Artemisinin (**21**), used for semi-synthesis of a common anti-malarial drug (Artemether), is also under investigation for cancer treatment. Other pharmaceutical crops are being cultivated for possible antiviral pharmaceuticals. For example, *Syzygium claviflorum* (Roxb.) Wall. ex A.M. Cowan & Cowan (Myrtaceae), from Southeastern Asia and Australia, produces betulinic acid (**22**) (Fig. **8**), a lupane triterpenoid used in the semi-synthesis of dimethyl succinyl betulinic acid (**23**) (Fig. **8**) currently in anti-AIDS clinical trials [24]. *Lomatium suksdorfii* J. M. Coult. & Rose (Apiaceae), from the Pacific northwest of the United States, is a major source of the anti-HIV (human immunodeficiency virus) coumarin suksdorfin (**24**) (Fig. **8**), with semi-synthetic analogs currently being tested in clinical trials.

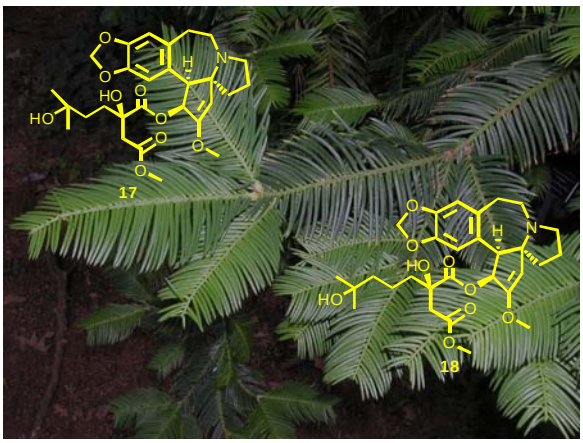

Fig. (5). Harringtonine (**17**) and homoharringtonine (**18**), two natural alkaloids isolated from *Cephalotaxus* spp. (*Cephalotaxus harringtonia* cultivated in Texas, USA, photo by S.Y. Li).

A number of pharmaceutical crops are being used to produce APIs for recently approved drugs for other diseases, such as Alzheimer's, although some of the drugs are also obtained synthetically. *Galanthus woronowii* Losinsk. (Amaryllidaceae) and related genera, including *Narcissus* L., are sources of galantamine (Razadyne®/Razadyne® ER, formerly known as Reminyl) (**25**) (Fig. **8**) [29]. This alkaloid is used for the treatment of mild to moderate Alzheimer's disease. The leaves of *Callistemon citrinus* Stapf. and the seeds of *Leptospermum scoparium* Forst. & Forst. (two shrubby species of the Myrtaceae from Australia and New Zealand) are the major source of the allelopathic essential oil leptospermone (**26**) (Fig. **8**); its analog mesotrione (**27**) (Fig. **8**) is used as a herbicide [30]. Recently, nitisinone (Orfadin®) (**28**) (Fig. **8**), a derivative of mesotrione, was the first drug approved in Europe for the treatment of hereditary type 1 tyrosinemia, a rare genetic metabolic disorder caused by a deficiency of the enzyme fumarylacetoacetate hydrolase (FAH) encoded by the FAH gene. This enzyme is involved in the metabolism of tyrosine [31].

Opium (*Papaver somniferum* L., Papaveraceae) is well known as a natural source of important alkaloids such as morphine (**29**) (a potent narcotic analgesic drug), thebaine (**30**), codeine (**31**) (an analgesic antitussive drug), and

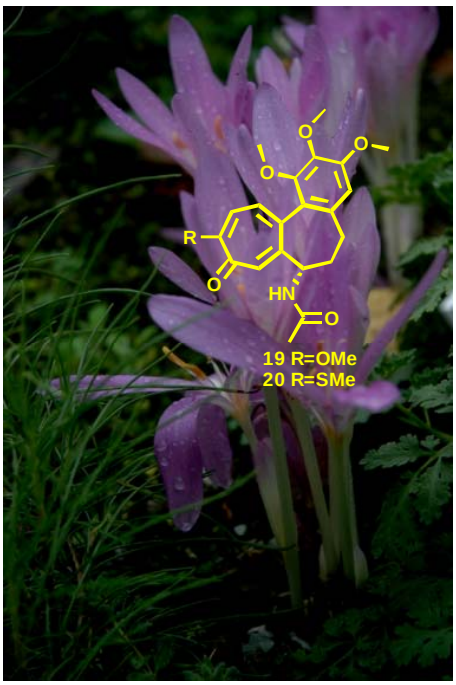

Fig. (6). Colchicine (**19**) and thiocolchicine (**20**), two natural alkaloids isolated from *Colchicum autumnale* (*Colchicum autumnale,* photo by M.J. Balick).

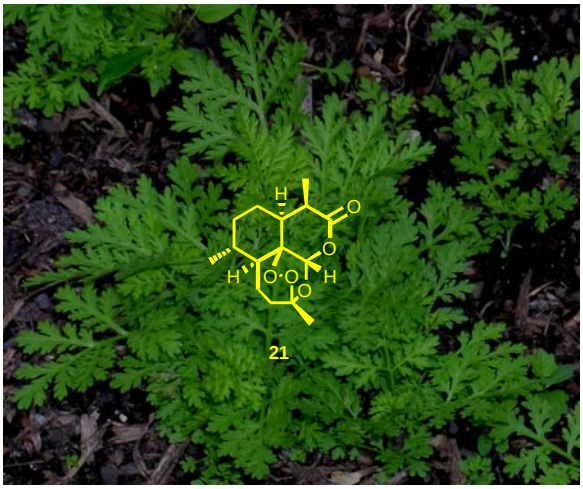

Fig. (7). Artemisinin (**21**) isolated from *Artemisia annua* (*Artemisia annua,* photo by M.J. Balick).

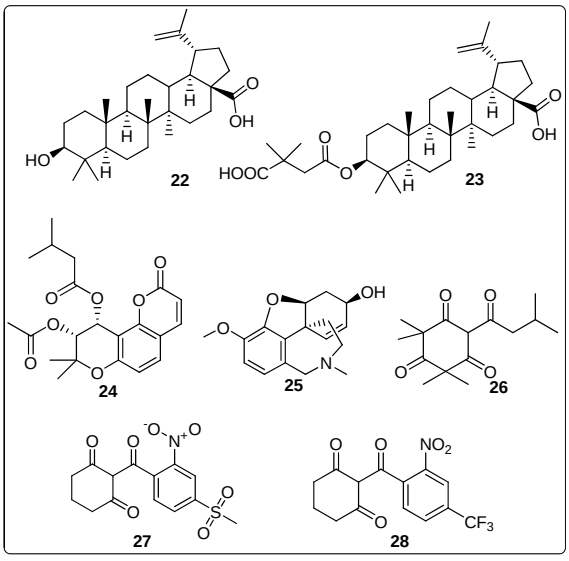

Fig. (8). Betulinic acid (**22**) isolated from *Syzygium claviflorum* and its semi-synthetic analog dimethyl succinyl betulinic acid (**23**), suksdorfin (**24**) isolated from *Lomatium suksdorfii*, Galantamine (**25**) from *Galanthus woronowii*, and leptospermone (**26**) from *Callistemon citrinus* and *Leptospermum scoparium* and its analogs mesotrione (**27**) and nitisinone (**28**).

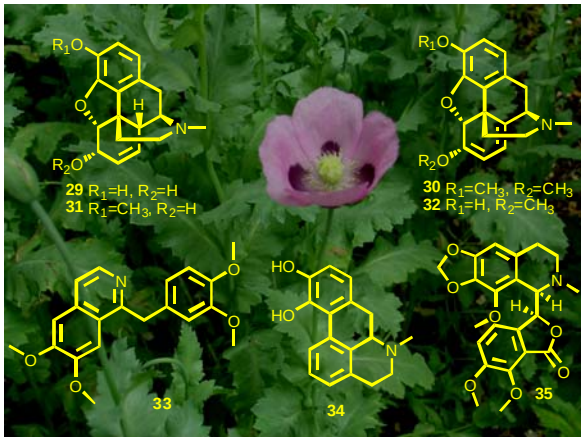

Fig. (9). Morphine (**29**), thebaine (**30**), codeine (**31**), oripavine (**32**), papaverine (**33**), noscapine (**35**), alkaloids isolated from *Papaver somniferum* and semisythetic apomorphine (**34**) (*Papaver somniferum,* photo by M.J. Balick).

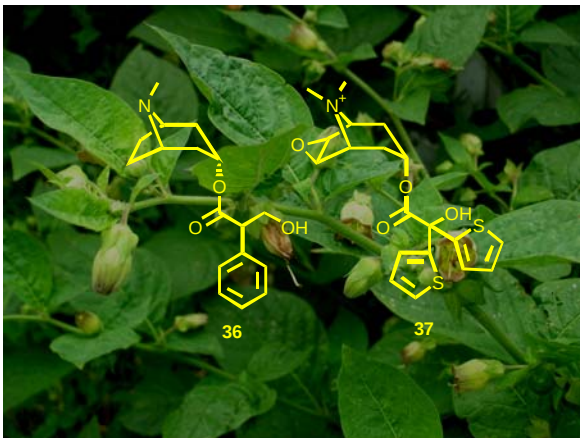

Fig. (10). Tropane alkaloids (−)-hyoscyamine (**36**) isolated from *Atropa belladonna* and semi-synthetic tiotropium (**37**) (*Atropa belladonna,* photo by M.J. Balick).

oripavine (**32**) (Fig. **9**). Papaverine (**33**) (Fig. **9**), another opium poppy alkaloid, is a smooth muscle relaxant used principally for the relief of cerebral and peripheral ischemia associated with arterial spasm and myocardial ischemia complicated by arrhythmias [32]. Apomorphine (**34**) (Fig. **9**), a semi-synthetic analog of morphine, was the first dopaminergic drug used to treat symptoms of Parkinson's disease [33]. Recently, noscapine (**35**) (Fig. **9**), another important alkaloid found in opium poppy, has emerged as a promising lead for chemoprevention and treatment of cancers especially prostate cancer, and stroke [34]. *Atropa belladonna* L. (Fig. **10**), *Hyoscyamus niger* L., and *Datura stramonium* L. (Solanaceae) are sources of (−)-hyoscyamine (**36**) (Fig. **10**) and atropine ((±)-hyoscyamine) which are used as anti-muscarinic agents. Anti-muscarinic alkaloids and synthetics are used in the treatment of a number of digestive disorders. They are also used to control excess motor activity of the gastrointestinal tract and spasm of the urinary tract, to dilate the pupils during ophthalmological examination of the eyes and in cases of iritis, reduce respiratory secretions in anesthesia, and nasal and sinus secretions in common cold and allergy [32]. A semi-synthetic tropane analog, tiotropium (**37**) (Fig. **10**), is used for treatment of bronchospasms associated with chronic obstructive pulmonary disease (COPD) [35]. Scopolamine or hyoscine (**38**), another tropane alkaloid found in *Datura metel* L. (Fig. **11**), is used for nausea and vomiting associated with motion sickness and for preanesthetic sedation with analgesics [32]. Some other important APIs are alkaloids ephedrine (**39**) (Fig. **12**) from *Ephedra sinica* Stapf (Ephedraceae), commonly known as ephedra or Ma Huang, a potent sympathomimetic bronchodilator for bronchial asthma and local treatment of nasal congestion [32, 36], physostigmine (**40**) (Fig. **12**) used for glaucoma and Alzheimer's disease from *Physostigma venenosum* Balf. (Calabar bean or ordeal bean; Fabaceae) [32, 37] and steroidal sapogenins diosgenin (**41**) (Fig. **12**) from *Dioscorea* spp. (yams; Dioscoreaceae) and hecogenin (**42**) (Fig. **12**) from *Agava* spp. (agaves; Agavaceae) for production of steroidal drugs [38, 39].

Other pharmaceutical crops produce inactive or less active pharmaceutical precursors used for synthesis of drugs. *Liquidambar styraciflua* L., known as sweetgum (Hamamelidaceae), is one of the most common hardwood species

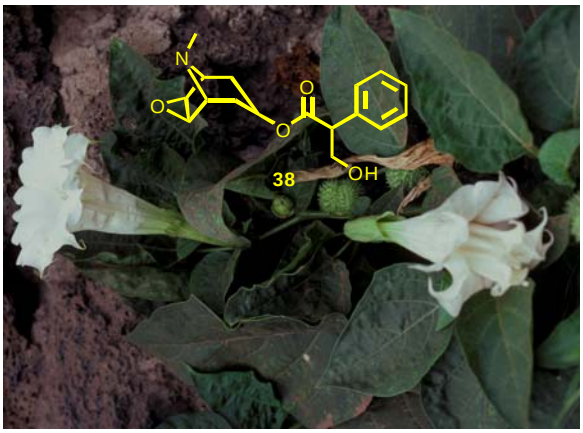

Fig. (11). Scopolamine (**38**) isolated from *Datura metel* (*Datura metel,* photo by R. Howard).

in the southeastern United States. Leaves of the tree contain up to 13.4% shikimic acid (**43**) (Fig. **13**) [40]. Shikimic acid (**43**) has weak bioactivity, but is a precursor for the antiviral drug Tamiflu®. Commercial harvest and extraction methods for high concentrations of stable pure shikimic acid (**43**) from the leaves have been developed [40], making *L. styraciflua* a promising pharmaceutical crop.

Pharmaceutical Crops for the Production of Large Therapeutic Molecules (LTMs)

Pharmaceutical crops for LTMs (usually having a molecular weight of more than 10,000 Daltons) include (1) crops producing endogenous LTMs such as proteins and polysaccharides, and (2) GM or engineered crops for producing exotic proteins such as vaccines and antibodies.

Plants produce a variety of bioactive proteins such as ribosome-inactivating proteins (RIPs), defensins, cyclotides, and lectins [41]. Some of these proteins have shown promising anti-cancer, antiviral, and antifungal activities and improvement of immune function in humans [41, 42]. Some important examples include antifungal and antiviral panaxagin from Asian ginseng (*Panax ginseng* C.A. Mey.; Araliaceae), quinqueginsin from North American *P. quinquefolius* L., trichosanthin and TAP 29 from *Trichosanthes kirilowii* Maxim. (Cucurbitaceae), Momordica Anti-HIV Protein (MAP30) from *Momordica charantia* L. (Cucurbitaceae), pokeweed antiviral protein (PAP) from leaves or seeds of

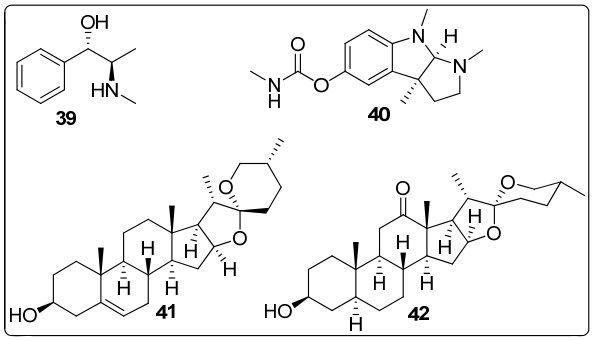

Fig. (12). Ephedrine (**39**) isolated from *Ephedra sinica*, physostigmine (**40**) from *Physostigma venenosum,* diosgenin (**41**) from *Dioscorea* spp., and hecogenin (**42**) from *Agava* spp.

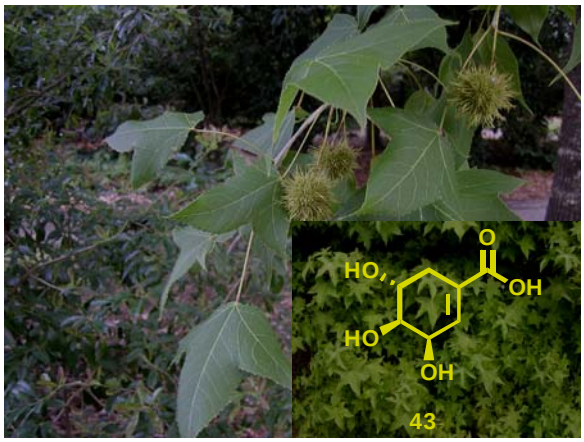

Fig. (13). Shikimic acid (**43**) isolated from *Liquidambar styraciflua* (*Liquidambar styraciflua* in East Texas, USA, photos by S.Y. Li).

Phytolacca americana L. (Phytolaccaceae), saporin found in seeds and leaves of *Saponaria officinalis* L. (Caryophyllaceae), gelonin from the seeds of *Gelonium multiflorum* A. Juss. (Euphorbiaceae), toxic ricin isolated from the castor bean (*Ricinus communis* L.; Euphorbiaceae), viscumin from *Viscum album* L. (Santalaceae), and abrin from seeds of *Abrus precatorius* L. (Fabaceae) [41-46]. Some proteolytic enzymes (proteases) isolated from plants are papain and chymopapain isolated from latex of papaya (*Carica papaya* L., Caricaceae), ficin from trunk latex of figs (*Ficus carica* L., *F. glabrata* Kunth; Moraceae), and bromelain from stem and fruits of pineapple (*Ananas comosus* (L.) Merr.; Bromeliaceae) [44].

Transgenic plants have been used for the production of antibodies directed against dental caries, rheumatoid arthritis, cholera, *E. coli* diarrhea, malaria, certain cancers, Norwalk virus, HIV, rhinovirus, influenza viruses, hepatitis B virus, and herpes simplex virus [47]. Protein antigens from various pathogens have been expressed in plants and used to produce immune responses resulting in protection against *Vibrio cholerae*, enterotoxigenic *E. coli*, hepatitis B virus, Norwalk virus, rabies virus, human cytomegalovirus, rotavirus, and respiratory syncytial virus F [47]. In 2006, production of the monoclonal antibody CB-Hep.1 (used in the manufacturing process for a Hepatitis B vaccine) in tobacco plants was approved in Cuba [48]. GM pharmaceutical crops could produce large quantities of drugs or vaccines at low costs. Production of therapeutic proteins by transgenic pharmaceutical crops usually has shorter development cycles [5] and lower cost than those from cell culture systems [47]. Like mammalian cells, plant production systems have the advantage over microbial systems of being able to produce active forms of complex proteins with appropriate post-translational modifications (e.g., glycosylation) [47]. Additionally, using pharmaceutical crops reduces the risk for unintentional transformation of viruses that infect humans as might occur when using mammalian cell systems.

Pharmaceutical Crops for the Production of Standardized Therapeutic Extracts (STEs)

Crops in this category are usually used in traditional medicine in various countries. It is estimated that several thousand species are used for production of STEs in the

world. Unlike STMs which are single molecular entities, STEs are a mixture of multiple active compound(s) extracted from pharmaceutical crops as standardized extracts. In this case the crops may be wild harvested, sometimes managed in local ecosystems, or cultivated in fields or environments such as agroforests. STEs can be mixtures extracted from one plant or from several to many different species. Many STEs are marketed and utilized in the same fashion as drugs in many parts of the world including China, Japan, India, Europe, and Africa. In the United States, STEs are usually classified as conventional foods and dietary supplements, depending on the specific claim as described in the Dietary Supplement Health and Education Act (DSHEA) of 1994. There has been an increasing interest in further development of STE drug products in recent years. In 2006, the FDA approved the first botanical drug Veregen® for the topical treatment of patients with perianal and genital condyloma [49]. The bioactivity of Veregen® is probably due to sinecatechins, a mixture of catechins found in the partially purified fraction of the water extract of green tea leaves from *Camellia sinensis* (L.) Kuntze (Theaceae) (**44**-**49**) (Fig. **14**), as well as other green tea components.

Probably less than 1,000 species are cultivated, with most of the species being harvested in the wild. With increasing demands and decreasing resources in the wild, however, more and more species are cultivated as crops for production of STEs. Some well-known examples are *Ginkgo biloba* L. (Ginkgoaceae), echinacea (*Echinacea angustifolia* DC., *E. purpurea* (L.) Moench, *E. palida* (Nutt.) Nutt; Asteraceae), liquorice (*Glycyrrhiza glabra* L.; Fabaceae), St. John's Wort (*Hypericum perforatum* L.; Clusiaceae), ginseng (*Panax ginseng* C.A. Meyer, Araliaceae), American ginseng (*Panax quinquefolius* L., Araliaceae), pines (*Pinus* L., e.g., *P. maritima* Miller., *P. sylvestris* L., *P. radiata* D. Don, *P. massoniana* Lamb.; Pinaceae) (pine bark extract, containing proanthocyanidins), and Reishi (lingzhi) (*Ganoderma tsugae,* Ganodermataceae).

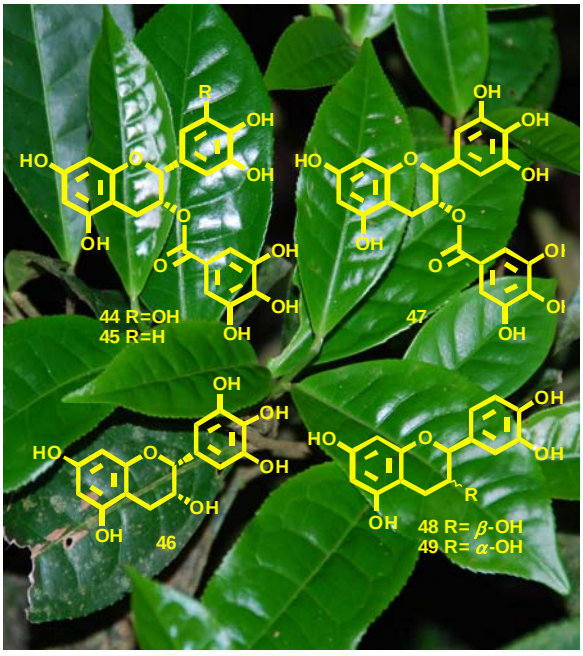

Fig. (14). Catechins isolated from green tea leaves of *Camellia sinensis*: (−)-epigallocatechin gallate (EGCG) (**44**), (−)-epicatechin gallate (ECG) (**45**), (−)-epigallocatechin (EGC) (**46**), (−)-gallocatechin gallate (GCG) (**47**), (+)-epicatechin (EC) (**48**), and (−)-catechin (**49**) (*Camellia sinensis,* photo by M.J. Balick).

STEs used as APIs could be a single class of active compounds or synergistic mixtures of several classes of bioactive compounds. Cardiac glycosides such as oleandrin (50) and digitoxin (51) (Fig. **15**) are compounds known to inhibit Na^+/K^+-ATPase activity and induce apoptosis [50, 51]. Members of this family of compounds have been in clinical use for many years for the treatment of heart failure and atrial arrhythmia [52]. Over the last ten years, emerging evidence has suggested that cardiac glycosides or cardiac glycoside containing botanical extracts have great potential for managing malignant diseases [53-57]. For example, Anvirzel®, a hot water extract of oleander (*Nerium oleander* L.; Apocynaceae) (Fig. **15**) contains oleandrin (50) as a principle cytotoxic component and has been evaluated in a Phase I clinical trial in the United States against refractory human cancers [50]. Additionally, the safety and anticancer response of the supercritical CO_2 extract of oleander is currently being evaluated in a Phase I trial at The University of Texas, M.D. Anderson Cancer Center. Ornamental oleander is now cultivated as a pharmaceutical crop in the United States.

Some pharmaceutical crops are also cultivated for production of both STEs and STMs, i.e., *Pueraria lobata* (Willd.) Ohwi (Fabaceae) for puerarin (**52**) (Fig. **16**),

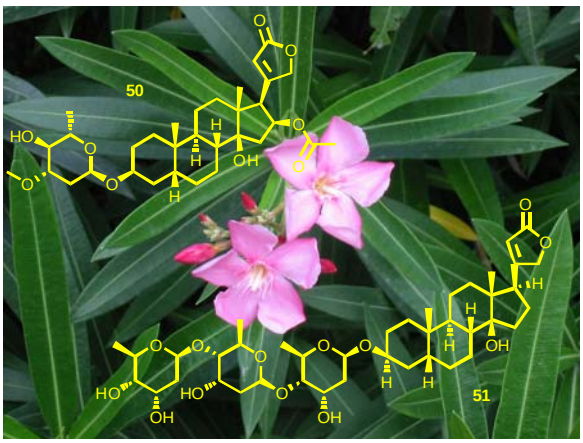

Fig. (15). Cardiac glycosides such as oleandrin (**50**) from *Nerium oleander* (photo by M.J. Balick) and digitoxin (**51**) from *Digitalis purpursea*.

Fig. (16). Puerarin (**52**) isolated from *Pueraria lobata* (*Pueraria lobata* in Oxford, Mississippi, USA, photos by S.Y. Li).

Lithospermum erythrorhizon Sieb. & Zucc. (Boraginaceae) for shikinon (**53**) (Fig. **17**), *Salvia miltiorhiza* Bunge (Lamiaceae) for tanshinone I (**54**), tanshinone IIA (**55**), salvianolic acid B (lithospermic acid B) (**56**), rosmarinic acid (**57**), cryptotanshinone (**58**), and neotanshinlactone (**59**) (Fig. **18**), and *Glycyrrhiza glabra* L. for glycyrrhizin (**60**) (Fig. **19**). *Salvia miltiorhiza,* one of the most popular plants used in traditional Chinese medicine, has been cultivated as a pharmaceutical crop in many Asian countries for the treatment of coronary heart disease, cerebrovascular disease, and inflammation [58, 59]. Although numerous varieties of *S. miltiorhiza* have been grown since the 1960s, a high-yielding cultivar is not yet available. The quality of APIs in cultivated *S. miltiorhiza* is much lower than that in wild populations

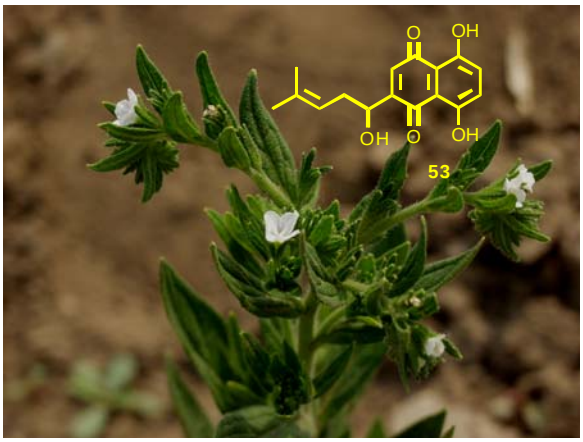

Fig. (17). Shikinon (**53**) isolated from *Lithospermum erythrorhizon* (photo by S.L. Chen).

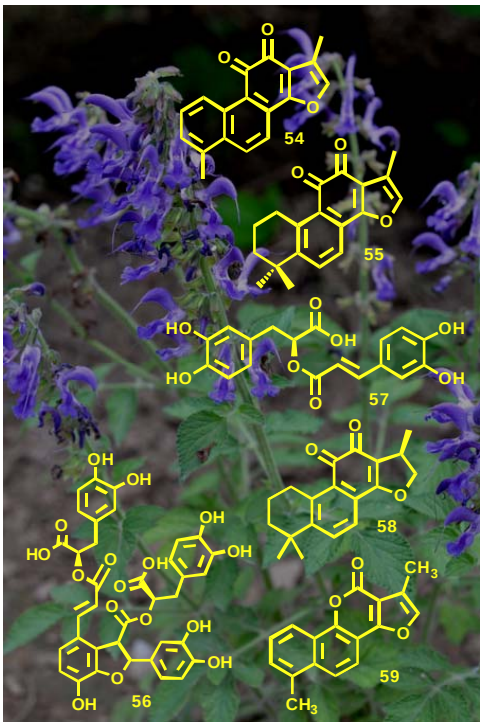

Fig. (18). Tanshinone I (**54**), tanshinone IIA (**55**), salvianolic acid B (lithospermic acid B) (**56**), rosmarinic acid (**57**), cryptotanshinone (**58**), and neotanshinlactone (**59**) isolated from *Salvia miltiorhiza* (photo by Y.L. Lin).

[60]. Although the harvested biomass rhizome production was increased significantly by applications of nitrogen fertilizers, the low and unstable yield of APIs has been a major problem with this species [58]. The medicinal potential of several other species of *Salvia* has been investigated [61, 62].

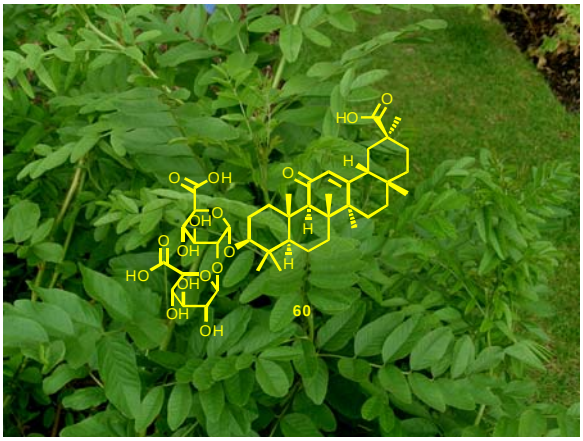

Fig. (19). Glycyrrhizin (**60**) isolated from *Glycyrrhiza glabra* (photo by M.J. Balick).

HOW PHARMACEUTICAL CROPS RELATE TO OTHER PLANTS AND CROPS

Pharmaceutical crops are a class of crops incorporating agriculture with medicine. Their relationships to medicinal plants, crops, medicinal crops, and traditional food crops are summarized in the Fig. **20**.

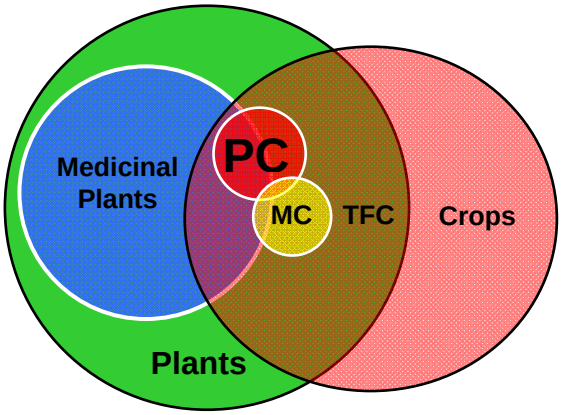

Fig. (20). Relationships of pharmaceutical crops (PC) to crops, medicinal plants, medicinal crops (MC), and traditional food crops (TFC).

Relationship to Medicinal Plants

There are over 70,000 plant species thought to be of medicinal value in the world [63]. Most of the medicinal plants in the world are still harvested in the wild and have not been developed as crops. In China, for example, there are approximately 31,000 plant species [64]. A total of 11,146 plant species have been recorded in Chinese herbal or medical literature according to a national survey conducted in 1995 [65]. Only 10% (1,200 species) are marketed as commercial medicines [66], of which 300 species are often cultivated as crops [58]. This trend is consistent with the use of the angiosperms as food; while over 3,000 are known to be used as foods only about 150 are in the economic system and

of those only a handful are major crops with global nutritional significance.

Most of the natural products in clinical use today were discovered through a routine examination of terrestrial plants and microorganisms [67]. Most pharmaceutical crops have their origins in previously known medicinal plants. However, some pharmaceutical crops emerged from the discovery, through established bioactivity-guided screens, of molecules in plants that have not previously been used in traditional medicine or considered as medicinal plants. Excellent examples of this include taxanes from *Taxus* and CPT from *Camptotheca*.

Relationship to Medicinal Crops

Many medicinal crops, originally cultivated for use in traditional medicine are now managed as pharmaceutical crops for STEs. For example, most of the 300 species commonly cultivated crops are currently also used for production of STEs in addition to their traditional uses. Other medicinal crops which are not used pharmaceutically, but which have a long history in folk medicine (herbal preparations) are only cultivated locally. These traditional medicinal crops could have potential as pharmaceutical crops in the future. As discussed above, some medicinal crops such as *Pueraria lobata, Lithospermum erythrorhizon, Salvia miltiorhiza,* and *Glycyrrhiza glabra* have been long cultivated as pharmaceutical crops for production of both STEs and STMs.

There is no clear definition for medicinal crops, the only criterion being that this class of cultivated species has some form of "traditional medicinal use". However, not all pharmaceutical crops have medicinal uses. As mentioned above, some food, vegetable, and spice crops are used for pharmaceutical purposes even though they are not typical medicinal crops. Likewise, some pharmaceutical crops are used as source of excipients (inert molecules) used in drug formulations as binders, fillers, disintegrants, emulsifying agents (e.g., gum Arabic and gum tragacanth).

Relationship to Traditional Food Crops

Some traditional food and vegetable crops are genetically modified or engineered to produce therapeutic proteins and thus are managed as pharmaceutical crops. In many cultures, medicinal diets have a long history together with cereals, vegetables, fruits, nuts, and spices which are used both as food and medicine. Over 2,000 plant species have been used as medicinal diets in China since the Tang Dynasty [68]. In the last several decades, standardized extracts or pure isolates from some traditional food crops have been developed as promising chemopreventive agents and even medicines [68].

Some traditional food crops have been developed as nutritional crops which are defined as sources for extraction or preparation of nutritional substances [69]. Nutritional crops can be also considered as pharmaceutical crops when some of their substances possess therapeutic properties. Grape (*Vitis* L.; Vitaceae) is a common nutritional crop. A number of studies suggest that grape seed extract (GSE, containing antioxidant oligomeric proanthocyanidins or OPCs) is useful for a variety of heart diseases and cancers. Many pharmaceutical products containing OPCs are available in the global market. Similarly, blueberries (*Vaccinium* L.; Ericaceae) are mainly cultivated for their nutritious berries, but the leaves are now also used for producing medicinal extracts [70]. Citrus fruits (*Citrus* L.,; Rutaceae) contain a variety of phytochemicals including flavonoids, limonoids, carotinoids, and vitamin C, and serve as a source of folic acid, high quality soluble fiber, and potassium [71]**.** Recent identification of the antiproliferative activity of limonoids (**61**) (Fig. **21**) from citrus against various human tumors [71-73] may lead to its development as a pharmaceutical crop.

Fig. (21). Limonoids (limonin (**61**)) isolated from citrus (*Citrus*) (photos by S.Y. Li).

Celery (*Apium graveolens* L. var. *dulce* DC.; Apiaceae) is an important vegetable crop. Apigenin (**62**) (Fig. **22**), a flavone isolated from celery which has been used to dye wool, has been shown to possess remarkable anti-inflammatory, antioxidant and anti-carcinogenic properties [74]. The cancer chemo-preventive effects of apigenin (**62**) have recently been reported [74].

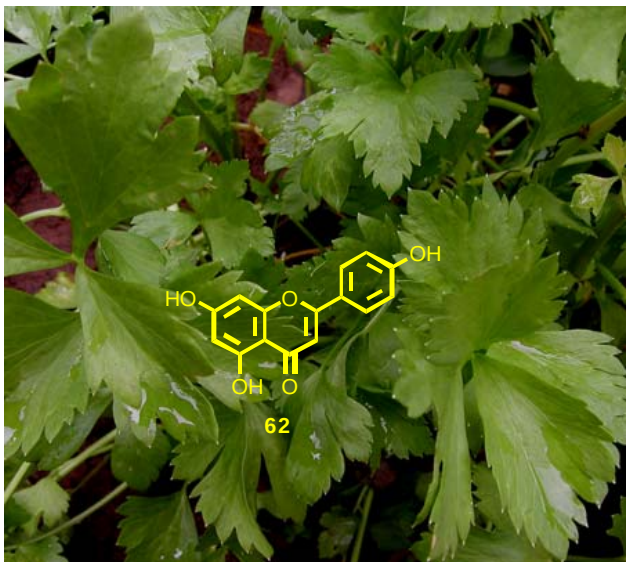

Fig. (22). Apigenin (**62**) isolated from celery (*Apium graveolens*) (photo by S.Y. Li).

Spice crops may also be cultivated as pharmaceutical crops. Tumeric (*Curcuma longa* L.; Zingiberaceae) (Fig. **23**) and star anise (*Illicium verum* Hook. f.; Illiciaceae) are two

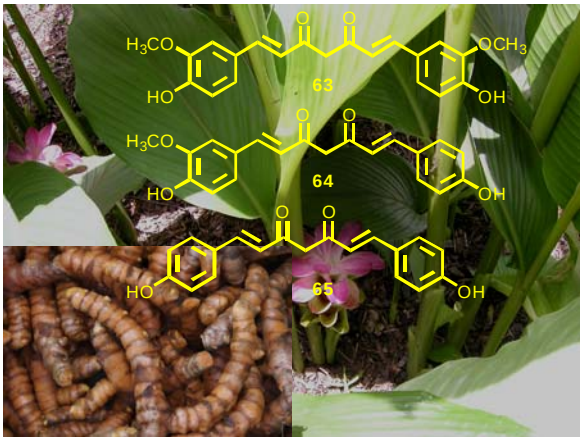

Fig. (23). Curcuminoids (curcumin (**63**), demethoxycurcumin (**64**), and bisdemethoxycurcumin (**65**)) isolated from tumeric (*Curcuma longa*) (photos by S.Y. Li and M.J. Balick).

common spices in Asia, but they are also cultivated for production of the pharmaceutical ingredients curcuminoids and shikimic acid (**43**), respectively. Curcuminoids, including curcumin (**63**), demethoxycurcumin (**64**), and bisdemethoxycurcumin (**65**) (Fig. **23**) have demonstrated anti-inflammatory and anticancer activity and promising potential in cancer prevention [75-77]. Aromatic plants not only serve as condiments and as important resources in the perfume and cosmetic industries, but have gained wide popularity now in aromatherapy [78, 79].

Relationship to Invasive Species

Some well-known invasive species in the United States such as kudzu or ge gen *(Pueraria lobata*), Japanese honeysuckle (*Lonicera japonica* Thunb.; Caprifoliaceae), and Japanese climbing fern (*Lygodium japonicum* (Thunb.) Sweet; Schizaeaceae) are currently managed as pharmaceutical crops in China and Japan. Kudzu is an invasive weed in the southeastern United States (Fig. **16**) which can completely replace existing vegetation. Eradication by herbicides can cost over $200 per acre for five years and thus is often commercially unfeasible [80]. In its native China, the vine is managed as a pharmaceutical crop to prepare extracts or isolate puerarin (**52**), or as a food crop. Puerarin (**52**) can induce angiogenesis in the myocardium and has been used to treat patients with cardiovascular diseases [81].

Each year, 3 million acres of land in the United States are taken over by invasive weeds. The spread of invasive plants is ranked second, behind habitat loss, as the greatest threat to biological diversity and ecosystem function in the United States. Controlling invasive species and the associated economic and environmental damages amounts to more than $138 billion per year [82]. More than $500 million is spent on residential exotic weed control and an additional $1 billion is invested in non-indigenous weed control on golf courses [82]. Efforts to control invasive species have been focused on "negative actions" such as herbicide or mechanical applications or biological control. A focus on ***utilization*** (positive actions such as identification of useful compounds in existing invasive species and subsequent development of these species as specialty or pharmaceutical crops valuable enough to stimulate removal/harvest and utilization—not replanting) rather than on ***elimination*** should be investigated as a possible approach to this problem in the United States and globally.

WHY PHARMACEUTICAL CROPS?

Plants as a Major Source of Drug Discovery

Plants have formed the basis for traditional medicine systems which have been used for thousands of years in China, India, and Egypt and later by the Greeks and Arabs. Botanical medicines still contribute to improving public health of the majority of the world's population [63, 83]. Plants have also proven to be a major source for the discovery of modern drugs, particularly in the cancer field [84]. Of 155 small molecules developed as anti-cancer drugs worldwide from the 1940s to the present time, 72.9% are naturally-inspired, with 47% being either the natural products or semi-synthetic derivatives [85]. Many well-known anti-cancer drugs are of plant origin, e.g., CPTs (**3-4**), taxanes (**5-7**), podophyllins (**10-12**), and vinca alkaloids (**13-16**) [67, 86]. Several experimental plant-based drugs have also shown promising potential: homoharringtonine (**18**) (alkaloid from *Cephalotaxus harringtonia*) for leukemia; 4-ipomeanol (a pneumotoxic analog of furan isolated from *Ipomoea batatas* (L.) Poir.) for lung cancer; elliptinium (a semi-synthetic analog of ellipticine from *Bleekeria vitiensi*s (Markgr.) A.C. Smith) for advanced breast cancer; and flavopiridol (a synthetic flavone derived from alkaloid rohitukine isolated from *Amoora rohituka* (Roxb.) Wight & Arn. and *Dysoxylum binectariferum* Hook. F. ex Bedd.) for colorectal cancer [87]. As Drs. Wall and Wani stated, "undoubtedly, there are other highly active natural products from plant, marine, and fungal sources as yet unknown which, when discovered, will have therapeutic utility. Cancer is not one, but several hundred diseases and will require many different types of agents" [18].

Plants are the obvious choice for future research of drug development because they contain an almost infinite variety of novel molecules [88]. Many compounds have very complex structures that are too difficult and expensive to synthesize on an industrial scale. The global market for botanical and plant-derived drugs is expected to increase from $19.5 billion in 2008 to $32.9 billion in 2013 [89]. However, insufficient supply of source material has been one of the major problems for bulk production of plant-based pharmaceuticals [90].

Pharmaceutical Crops as a Solution to Secure Sustainable Supplies of Quality Medicine

Of the world's total flora of higher plants (estimated between 215,000 and 500,000 species), only 6% have been screened for biological activity and 15% evaluated phytochemically [91, 92]. Actually, far fewer species have been thoroughly screened or chemically investigated. Plants have been proven and will continue to provide novel leads for drug development [84, 90, 93-97]. Due to habitat destruction, overharvest, lack of sustainable utilization and conservation programs, however, species diversity and genetic diversity of medicinal plants are continuously under the threat of extinction. It has been estimated that 22-46% of the world's total plant species are endangered [98].

Many medicinal plants are being destroyed at an unprecedented rate and are threatened with extinction [63]. As Balick and Mendelsohn pointed out, the value of sustainably harvesting locally used medicinal plants in a forest plot in Belize far exceeded the use of that same land for agriculture [99]. The disappearance of medicinal plants, in addition to reducing the access of a local population to primary health care remedies, also threatens future efforts in drug discovery. At present, of the 70,000 species of medicinal plants in the world still primarily harvested in the wild, most have not been cultivated as crops. For example, of the 3,000 species of medicinal plants being traded in the world, 70-80% originate from wild-collections [100, 101], and only 900 have commercial cultivation underway or in development [102]. Some 15,000 medicinal plant species may be threatened with extinction [63].

Over-harvesting and loss of habitat often directly threaten medicinal plants. Current supplies of many critical pharmaceuticals having global demands still rely on wild collection. For example, *Taxus* tree species in North America and China were harvested for bark and needles to produce the cancer drugs paclitaxel (**5**) and docetaxel (**6**) and they are now in endangered status. Cultivation of *Taxus* species has now become essential to supplying these compounds. *Hoodia gordonii* Sweet ex Decne (Asclepiadaceae) in Southern Africa and Namibia and some other species of the genus are sources of weight loss drugs [103]. *Hoodia gordonii* is now included in the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II list in an attempt to control over-harvesting [103], and again, cultivation of *Hoodia* is now underway.

Over 2,000 medicinal and aromatic plants are traded commercially in Europe, and it is estimated that around 150 plant species are threatened in at least one European country by this trade [104]. *Colchicum autumnale* L. (Colchicaceae) used for gout is an example. *Arnica montana* Lam. (Asteraceae) and *Gentiana lutea* L. (Gentiaceae) are harvested throughout Europe (especially in Bulgaria and Romania) but are becoming scarce.

In China, of 354 species on the Chinese Endangered Plant Species List, 168 species are medicinal plants [105]. Over 30 species often used in Chinese medicine are endangered and in short supply, e.g., ginseng, *Glycyrrhiza glabra*, *Angelica sinensis* (Oliv.) Diels (Apiaceae), *Ligusticum chuanxiong* S. H. Qiu, Y. Q. Zeng, K. Y. Pan, Y. C. Tang & J.M. Xu (Apiaceae), *Dysosma versipellis* (Hance) M. Cheng ex T. S. Ying (Berberidaceae), *Changium smyrnioides* H. Woff (Apiaceae), *Actinidia macrosperma* C. F. Liang (Actinidiaceae), as well as three well-known woody medicinal plants the bark of which is used namely, *Magnolia officinalis* Rehder & E. H. Wilson (Magnoliaceae), *Phellodendron amurense* Rupr. (Rutaceae), and *Eucommia ulmoides* Oliv. (Eucommiaceae) [105, 106].

Extinction of wild plants will not only thwart the discovery of new drugs, but also limit the sustainable supply of quality plant-based pharmaceuticals. In China, the decreasing genetic pool of wild plants has resulted in quality problems with products from over 100 species frequently used for medicine [105]. Furthermore, indigenous knowledge of the value of many medicinal plants is being lost very quickly, although the vast majority of the earth's plants have yet to be thoroughly analyzed for their potential to improve healthcare [103]. Ethnobotanical research has an important role to play in drug discovery through establishing an inventory and then helping local people to conserve this aspect of traditional knowledge [107-109].

Although the disappearance of many medicinal plants is primarily the result of over-harvesting and loss of habitat, the lack of sustainable agriculture systems and conservation practices designed to maintain both biological and cultural diversity threatens the future use of medicinal plants and thus drug discovery programs. Current efforts such as *in situ* and *ex situ* conservation strategies which include cultivation in botanical gardens will preserve some endangered medicinal plant germplasm, but this should also include understanding and preservation of the knowledge of their value. Development and cultivation of pharmaceutical crops, however, will not only conserve endangered medicinal biodiversity, but will also provide sustainable production of therapeutic molecules. In addition, pharmaceutical crop research will help to conserve and utilize decreasing biological and cultural diversity and thus enhance the potential for drug discovery.

Development of pharmaceutical crops will secure supplies of vital medicines particularly important in emergent cases such as threat of pandemic infectious diseases. Some countries must currently import APIs to produce antiviral and anti-cancer drugs, and foreign supplies may be uncertain. Cultivation of newly emerging pharmaceutical crops in these locations may open the door to less expensive and more readily available drugs, and revitalize rural economies by establishing high-value crops.

Problems and Challenges with Pharmaceutical Crop Development

At present, when compared to research and development of food and fiber crops, similar programs to address pharmaceutical crops for therapeutic are very limited. Unlike traditional food crops and yet-to-be-developed energy crops for biomass, research in pharmaceutical crops focuses on the maximization and sustainable production of target chemical compound(s) from the plants. This involves the integration of techniques used in traditional crops including plant breeding, physiology and ecology, coupled with research in the fields of pharmacognosy, pharmacology, and eventually clinical studies. Integration of these different areas with interdisciplinary teams of scientists working towards a common goal appears to be the key to success.

The discoveries of CPT (**1**) and Taxol® (**5**) by Drs. Wall and Wani "firmly established the fact that the close cooperation between chemists and botanists was required for a successful natural products program" [18]. In search of novel bioactive natural products, chemists commonly start with dried plant parts (or tissues) provided by botanists and may never see the living plants. In contrast, botanists and plant physiologists are primarily focused on the content variations or biosynthesis of target compounds in intact plants or cell culture systems. Both chemists and biologists realize that inducible biosynthesis of target compounds will require extensive efforts in *in vitro* production and microbial biotransformation of these compounds. However, management of intact plant systems as reactors for derivatization of novel

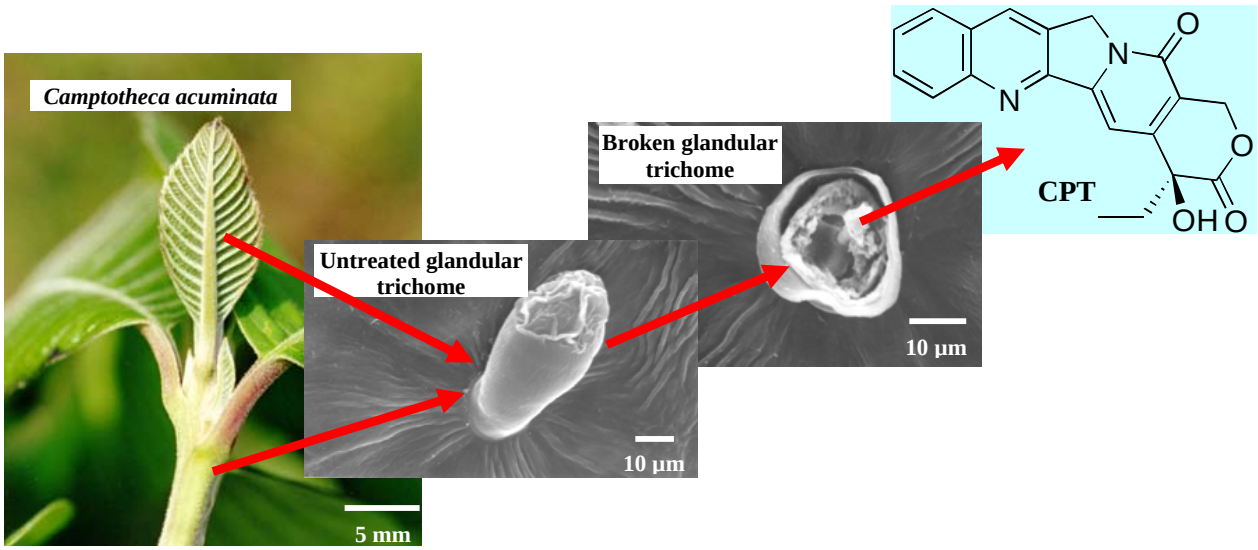

Fig. (24). "Trichome Management" method to induce biosynthesis and derivatization of camptothecin (CPT) in *Camptotheca acuminata*: glandular trichomes on the leaf and stem surfaces as accumulation sites of CPTs are management target in selection and cultivation of high-yielding variety [14, 16, 103] (photos by S.Y. Li and K. Northrup).

bioactive agents has never been seriously addressed. As a matter of fact, important secondary metabolites such as morphine are produced in significant amounts only in the intact plant, with the biosynthetic pathway being repressed in cell culture. Pharmaceutical crops are platforms for biologists, chemists, pharmacists, health professionals and clinicians to collaborate closely in making new drug discoveries in all disease areas. Several problems have tempered both industrial and academic enthusiasm for research and funding in pharmaceutical crops as a production system, particularly for the production of STMs in great global demand. In addition to decreasing genetic diversity in nature and environmental concerns (i.e., endangered species), factors including slow plant growth, long harvest cycle, and low and unstable yields of desired compounds in wild plants have been major problems in the large scale management of crops and production of the pharmaceuticals. Often, the yield and quality of pharmaceuticals from cultivated plants is not equivalent to those in the wild, even in common crops with a long cultivation history, such as *Salvia miltiorhiza* [58, 60]. Therefore, priority research in both intact plant and cell culture (when feasible) should be carried out to select and develop high and stable yielding varieties or strains having the desired target compound(s).

Future pharmaceutical crop research efforts should also emphasize the strategies to enhance the yield of therapeutic molecules or precursors in sustainable crop production systems. To obtain a diversity of bioactive analogs, methods need to be developed to increase derivatization of the desired compounds in intact plants or *in vitro* systems, in addition to exploring microbial biotransformation. In some cases, STMs or precursors accumulate in glandular trichomes of plants. The shape and size of glandular trichomes from different species within a genus may vary considerably but are highly heritable [14, 16]. Induction of the production of glandular trichome in plants could significantly increase the yield of the target compounds. In the 1990s, the "Trichome Management" technique was developed to increase both biomass of leaves and stems and the density of glandular trichomes on leaves and stems by controlling auxin levels [14, 16] (Fig. **23** to Fig. **24**). This method induced production of glandular trichomes and thus increased alkaloid CPT yield by 16 to 20-fold, and derivatization of CPT in *Camptotheca* [14, 16, 110, 111]. The alkaloidal induction by auxin reduction in *Camptotheca* consists of two steps: an emergent response to translocate alkaloids from old to young tissues and a systematic response to increase alkaloidal contents in the entire plant [16]. Recently, trichome density was used as a trait to quickly screen and develop high-yielding varieties of *Artemisia annua* for sesquiterpenoid artemisinin yield [112]. Although both trichome density and secondary metabolites may be inducible by auxin-reducing pruning [16, 40, 110], the induction mechanism remains elusive. Existing hypotheses attempting to explain the increase in secondary metabolite concentration after damage are conflicting (supply-side vs. demand-side); but both emphasize the defense function of secondary metabolites, particularly of phytoalexins [113, 114]. However, the role of defense in induced biosynthesis of toxic compounds in plants needs further investigations. The chemicals produced by *Taxus* spp., *Artemisia annua, and Camptotheca* spp. are among the most extensively investigated. There is no information on whether protection from herbivores or pathogens occurs in *Taxus* or *Artemisia* [115]. In *Camptotheca*, elevated toxic CPTs did not increase the antifungal activity of the plants but actually induced plant endogenous autotoxicity [110]. Thus, a chemical defense role should not be overemphasized in the induced biosynthesis of some toxic secondary metabolites. Another interesting topic worthy of investigation is the cyclical nature of plant chemical levels within individuals of the same species. For example, in *Hypericum perforatum*, levels of hypericin and pseudohypericin were found to vary between 100 ppm and 5,000 ppm from winter to summer [116].

Selection of optimum or suitable zones for crop cultivation is important to the development of high-yield and sustainable production systems, particularly for crops with long

harvest cycles. The process involves analysis of multiple factors in ecology, climatology, geology, biology, and chemistry. Recently, some GIS (Geographic Information System)-based models for adaptability analysis were established [117]. Some regional zoning efforts have also been made with several common traditional medicinal crops in China [117]. In non-native ranges, conducting field trials over large regions are time and cost prohibitive and could limit cultivation of crops in new areas.

The difficulty in isolating and characterizing complex natural products is becoming less of a problem as HPLC-MS and HPLC-NMR are more routinely used and new methods for isolation and structural elucidation continue to be developed [90]. However, purification of some desired compounds from pharmaceutical crops, and particularly the elimination or reduction of interfering compounds in plants continues to be a major challenge in extraction. Effective isolation of desired compounds in intact plant systems needs to be further investigated.

In recent decades there have been great efforts and significant progress in exploring other options to produce therapeutic compounds. Although chemical synthesis is a commercially feasible option for those compounds with relatively simple structures such as aspirin and ephedrine, it is not an appropriate strategy for complex structures [118-120]. Semi-synthesis from more abundant natural precursors is often necessary to prepare structurally complex drugs, e.g., semi-synthesis of taxane-based drugs (**5-7**) from natural baccatins isolated from *Taxus* spp. [22, 23]; also semi-synthesis is often used to convert toxic natural products to derivatives having more acceptable pharmacological properties, e.g., the conversion of CPT (**1**) to topotecan (**3**) and irinotecan (**4**).

Metabolic engineering of target secondary metabolites in plants is feasible, but requires knowledge of the biosynthetic pathways [121]. The secondary metabolic pathways of only a few plants have been studied in detail which limits progress in this area [119, 121]. *Catharanthus roseus* and *Rauvolfia serpentina* Benth. ex Kurz (Apocynaceae) have been used as model systems for the investigation of monoterpene indole alkaloid production by *in vitro* culture techniques, particularly cell suspension cultures, since the early 1980s [119]. However, *C. roseus* and *R. serpentina* crops are still the only viable commercial source of the anti-cancer vinca alkaloids and anti-hypertensive *Rauwolfia* alkaloids [119, 122, 123]. Successful examples of secondary metabolite production by plant cell suspension cultures include the anti-cancer paclitaxel (**5**) from *Taxus* spp.; the anti-cholinergic scopolamine (**38**) from *Duboisia* spp. (Solanaceae); the antibiotic berberine from *Coptis japonica* Makino (Ranunculaceae); the anti-inflammatory rosmarinic acid (**57**) from *Coleus blumei* Benth. (Lamiaceae); and the anti-inflammatory and antiviral shikonin (**53**) from *Lithospermum erythrorhizon* Siebold et Zucc. (Boraginaceae) [124]. Other options including expression of plant pathways in microbial systems have been explored. The commercial potential of hairy root cultures for production of plant-derived pharmaceuticals has been limited primarily due to challenges in cultivating hairy roots in a large scale system [120]. Research and development in these fields will definitely enhance the pivotal role of pharmaceutical crops. In general, however, the use of intact plant systems means lower cost of production and easier expansion for large-volume production [47]. Instead of a large capital investment in cell culture facilities, plant production systems can be expanded simply by growing and harvesting additional plants.

Conservation biologists and ecologists have raised concerns about the biosafety and environmental impacts of GM pharmaceutical crops. The potential environmental impacts (e.g., gene flow between cultivated crops and their wild relatives) of genetically engineered crops for pharmaceuticals need constant analysis and evaluation. However, the use of biotechnology in pharmaceutical crops and conservation of biodiversity are not mutually exclusive.

Another issue is reciprocity to indigenous cultures and people that contributed knowledge and resources for development of pharmaceutical crops and their products. To ensure that more equitable benefits accrue from the use of biological resources, the United Nations Convention on Biological Diversity (CBD) was drafted during the Earth Summit in Rio de Janiero in 1992 [125]. Since then, there has been an increasing awareness in developing countries of the potential value of their indigenous genetic resources [126]. In fact, governments and conservation biologists have made extensive efforts to ensure the sustainable use of biodiversity and benefit sharing from revenues generated from plants originally used by indigenous and local people in traditional medicine. Shaman Pharmaceuticals developed a model for the pharmaceutical industry to have multi-stage benefit sharing with governments and traditional cultures in its drug discovery from Amazonian plants [125, 127], and the National Cancer Institute produced a series of agreements to address this issue that are also used as models by others [126]. Recently, some have argued that all nature-based patents should acknowledge evolution by natural selection as a 'co-inventor' and should allocate 1% of patent ownership towards efforts to conserve biodiversity [128].

CONCLUSIONS

Pharmaceutical crops can be defined as cultivated species that are used for the extraction or preparation of therapeutic substances such as active pharmaceutical ingredients (APIs), excipients used in pharmaceutical formulations, vaccines and antibodies, as well as other therapeutic proteins. Basically, there are three types of pharmaceutical crops: crops for the production of small therapeutic molecules (STMs), large therapeutic molecules (LTMs), and standardized therapeutic extracts (STEs). This review focuses on examples of terrestrial plants although pharmaceutical crops could be either terrestrial or aquatic species. Pharmaceutical crops are a relatively new class of crops having close relationships with medicinal plants, medicinal crops, and traditional crops. Unlike traditional food crops and yet-to-be-developed energy crops for production of biomass, research in pharmaceutical crops focuses on the maximization and sustainable production of target chemical compound(s) from the plants. This involves the integration of techniques used in traditional crops including plant breeding, physiology and ecology, coupled with research in the fields of pharmacognosy, pharmacology, and eventually clinical studies. Successful development of pharmaceutical crops requires extensive joint efforts of scientists in these different fields. Research in the area of invasive species has been focused on elimination, but

the potential for the development of some species as pharmaceutical crops needs to be exploited. Future development of pharmaceutical crops should emphasize strategies to enhance the yield and derivatization of therapeutic molecules or precursors in sustainable crop production systems, including development of high-yielding cultivars, cultivation technology for induced production of desired compounds, and effective and environmental-friendly extraction methods. In addition, biosafety, environmental impacts, and benefit sharing should be addressed in the research and development of pharmaceutical crops.

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ABBREVIATIONS

API	=	Active pharmaceutical ingredient
CBD	=	The United Nations Convention on Biological Diversity
CITES	=	Convention on the International Trade in Endangered Species of Wild Fauna and Flora
CPT	=	Camptothecin
COPD	=	Chronic obstructive pulmonary disease
10-DAB	=	10-deacetylbaccatin III
DSHEA	=	Dietary Supplement Health and Education Act
FAH	=	Fumarylacetoacetate hydrolase
FDA	=	The United States Food and Drug Administration
GIS	=	Geographic information system
GM	=	Genetically modified
GSE	=	Grape seed extract
HCPT	=	10-hydroxycamptothecin
HIV	=	Human immunodeficiency virus
LTM	=	Large therapeutic molecule
MAP30	=	Momordica anti-HIV protein
OPC	=	Oligomeric proanthocyanidin
PAP	=	Pokeweed antiviral protein
RIP	=	Ribosome-inactivating protein
STE	=	Standardized therapeutic extract
STM	=	Small therapeutic molecule
TOPI	=	DNA topoisomerase I

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