PLANTS AS SOURCE OF ANTI-CANCER AGENTS: A BRIEF REVIEW

Tej Prakash Singh*, Amit Gupta, Pramod KumarSharma, Sombhu Charan Mondal

Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Baghpat Bypass, Delhi-Haridwar HighwayNH-58, Meerut-250005(U.P).
Email: tej.prakesh203@gmail.com
Contact no: +91 9891600249

Summary

Cancer is a major concern in both developed and developing countries after cardiovascular diseases. It is characterized by abnormal growth of cells. A large number of anticancerous agents derived from plants are being used for the treatment of cancer from ancient time. Several anticancer agents of plant origin including taxol, vinblastine, vincristine, topotecan and irinotecan, camptothecin derivatives and etoposide derived from epipodophyllotoxin are in clinical use all over the world. A number of promising agents such as flavopiridol, roscovitine, combretastatinA-4, betulinic acid and silvestrol are in clinical or preclinical development. This review consist brief description of plants having anticancerous properties.

Key words: anticancer, cytotoxicity, natural products, medicinal herbs, alternative medicine, cancer.

Introduction

Medicinal herbs are significant source of synthetic and herbal drugs. Before the availability of synthetic drugs, man was completely dependent on herbs as healthcare system. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and a large number of modern drugs have been developed from them. Today Chinese herbal medicine occupies second position in world. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumeriansand Akkaidians [1]. The "Ebers Papyrus", an Egyptian pharmaceutical record, documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 BC. The Chinese *Materia Medica,* which describes more than 600 medicinal plants, has been well documented with the first record dating from about 1100 BC [2].

Documentation of the Ayurvedic system recorded in Susruta and Charaka dates from about 1000 BC [3]. The Greeks also contributed substantially to the rational development of the herbal drugs. Dioscorides, the Greek physician (100 A.D.), described in his work "De Materia Medica" more than 600 medicinal plants [1]. The World Health Organization estimates that approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care [4]. Cancer is a major public health burden in both developed and developing countries. It was estimated that there were 10.9 millions new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002 [5]. Cancer is the second leading cause of death in the United States where one in four deaths is due to cancer [6]. Plants have long been used in the treatment of cancer [7]. The National Cancer Institute collected about 35,000 plant samples from 20 different countries and has screened around 114,000 extracts for anticancer activity [8]. About 92 anticancer drugs were commercially available prior to 1983 in the US and among world wide approved anticancer drugs between 1983 and 1994, 60% are of natural origin [2]. In this instance, natural origin is defined as natural products, derivatives of natural products or synthetic pharmaceuticals based on natural product models [9].

Brief History

Natural products of plant origin were major source in ancient Indian medicinal science.Recently, numerous bioactive compounds have been isolated from plant sources and several of them are currently in clinical trials. Plant-derived compounds have been a vital source of varied clinically useful anti-cancer agents such as camptothecin derivatives, topotecan and irinotecan. Furthermore, other potent molecules include vinca alkoloids (vinblastine, vincristine), flavopiridol, a semi-synthetic analogue of the chromone alkaloid and rohitkine, a pyridoindole alkaloid derived from leaves of Ochrosia species. Presently, anticancer drug development research is mainly dependent on exploring potential phytochemicals because of cost cutting factor. Being a tropical country Indian sub-continent is rich in its diversity of flora, with a large spread of rain forests and river basins. It is floristically rich with about 33 percent of its botanical wealth (over 15,000 species of higher plants) being endemic. Different classes of compounds having anticancerous properties are shown in Table-1.

Table- 1: Different class of compounds having anticancerous properties

Class	Plant sources	Compounds
bis- indol	*Catharanthus roseus and C.lanceus, C.roseus* (Apocynaceae) *C.roseus* (Apocynaceae)	Leurosine Vinblastine Vincristine
Monoterpine	*Allamanda Cathartica Ipomea Batatas*	Allamandine 4-Ipomeanol
Diterpene	*Taxus brevifolia Taxodium distinchium Jatropha gossypiifolia*	Taxol Taxodione Jatrophone
Lignan	*Podophyllum hexandrum Podophyllum peltatium Podophyllum peltatin*	Podophyllotoxin Alpha and Beta-peltatin
Flavonoid	Silybium marianum (Asteraceae)	Silymarine silydianin

Herbal Product As anticancer agents in clinical use

Catharanthus roseus, family Apocynaceae a tropical perennial plant produces more than 100 monoterpenoid indoles. The isolation of two commercially important cytotoxic dimeric alkaloids like vinblastine and vincristine from the Madagascar periwinkle plant introduced a new era of plant derived anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer [10]. Vinblastine and vincristine alkaloids are mainly used in combination with other cancer chemotherapeutic agents for the treatment of cancers, including leukemias, advanced testicular cancer, lymphomas, Kaposi's sarcoma, breast and lung cancers [10].

Paclitaxel obtained from the bark of the pacific yew tree (*Taxus brevifolia*, family-Taxaceae) is evidence of the success in natural product drug discovery. It is used in the treatment of breast, ovarian and non-small-cell lung cancer. Various parts of *Taxus brevifolia* and other taxus species (e.g., *Taxus Canadensis* Marshall, *Taxus baccata* L.) have been used by several Native American tribes for the treatment of some noncancerous cases [10], while *Taxus baccata* was reported to use in the Indian ayurvedic medicine for the treatment of cancer. In the early 1990s paclitaxel was clinically introduced to the US and its structure was elucidated in 1971 [11].

Camptothecin isolated from the Chinese ornamental tree *Camptotheca acuminate* Family Nyssaceae, was advanced to clinical trials by national cancer institute in the 1970s but was dropped because of severe bladder toxicity [12]. Topotecan and irinotecan are semi-synthetic derivatives of camptothecin and are used for the treatment of ovarian and small cell lung cancers, and colorectal cancers respectively [13].

Camptotheca acuminate is the source of four promising anticancer drugs, two of which have been approved by the FDA. The other two chemicals still under research include: 9AC (9-aminocamptothecin): Currently in clinical trials for several types of cancer, including ovarian and stomach cancers and T-cell lymphoma. Irinotecan is another chemical analog which has been developed from another plant alkaloid discovered in the same tree, *Camptotheca acuminata*, for the treatment of metastatic colorectal cancer.

Epipodophyllotoxin is an isomer of podophyllotoxin which was isolated as the active anti-tumor agent from the roots of *Podophyllum* species, *Podophyllum peltatum* L and *Podophyllum emodi* W (Berberidaceae). Etoposide [8] and teniposide [9] are two semisynthetic derivatives of epipodophyllotoxin and are used in the treatment of lymphomas in bronchial and testicular cancers. Teniposide is a semisynthetic derivative of a plant chemical discovered in the Mayapple plant family (*Podophyllum peltatum*).

Homoharringtonine, isolated from the Chinese tree *Cephalotaxus harringtonia* var. *drupacea* (Cephalotaxaceae), is another plant-derived agent in clinical use [14]. A racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia [11].

Elliptinium a derivative of ellipticine, isolated from a Fijian medicinal plant *Bleekeria vitensis,* is marketed in France for the treatment of breast cancer [15].

The essential oils isolated by hydro-distillation from *Pelargonium graveolens* (Geranium) freshed aerial parts (stem, stalks, leaves) and essential oils isolated by hydro-distillation are used for their anticancerous property.

Lapchol isolated from heartwood of *Tabebuia impetiginosa*, *Tabebuia avellanedae* (Bignoniaceae), commonly known as Lapacho tree used for its anticancerous activity. The component which shows anticancerous activity was 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-napthoquinone [10].

Flavopiridol is a synthetic flavone derived from the plant alkaloid rohitukine, which was isolated from the leaves and stems of *Amoora rohituka* and later from *Dysoxylum binectariferu.* Flavopiridol present inhibit cyclin dependent kinase. The agent is currently in phase I–II clinical trials. Available evidence indicates encouraging response rates in a variety of solid and haematological malignancies and diarrhoea as the dose-limiting toxicity [10].

Citrus reticulate/ Petitgrain mandarin (Rutaceae) leaves collected from Behira, Egypt are used for their anticancer activities. *Petitgrain mandarin* essential oil and the major components like ɣ-terpinene (47.89%), methyl N-methyl anthranilate (13.17%), α-terpinene (7.40%), β-phellandrene (6.26%) and trans isolimonene (5.87%). On the other hand, Thirty two compounds constituting 99.23% of geranium essential oil have been identified for their anticancerous activity.

Curcuma longa obtained from the root and rhizome of plant known as *Curcuma longa*, family Zingiberaceae. In china *C. wenyjuin* (*C. aromatica*) has been used in cervical cancer. Chief constituent beta elemene is used in cervical cancer [16-19].

Lycobetain a phenanthridine alkaloid obtained from various species of Amayllidaceae family. It act as selective topoisomerase poison and inhibit the growth of human tumour cells. Used in a number of ascites tumour [18-19].

Cephalotaxus alakloids was obtained by extraction from the Chinese evergreen tree *Cephalotaxus harringtonia* K var. harringtonia [20].

Ipecacuanha is obtained from the dried roots and rhizome of *Cephalis ipecacuanaha* or *Cephalis acuminate* family Rubiaceae. Chief constituent emetin have antitumour property.

The extract and isolated diterpenes (andrographiside and neoandrographolide) from *Andrographis paniculata* are proved to be beneficial against tumourigenesis by their anti-lipoperoxidative action and by enhanced carcinogen detoxification action [21-24].

Colchicine originally extracted from plants of the genus Colchicum (Autumn crocus, *Colchicum autumnale*, also known as "Meadow saffron". It is also being investigated for it anticancerous activity.

An alkaloid called piperine obtained from extract of *Piper longum* is used in anticancer formulations [25].

An aqueous extract of *Phyllanthus niruri/P. amarus* increases the life span of the tumour bearing rats and normalizes glutamyl transpeptidase activity. It plays a major role in disruption of HBs Ag mRNA transcription and post-transcription which could be beneficial against viral carcinogenesis [8, 10].

(+)-Calanolide A and (-)-Calanolide B (costatolide) are isolated from tree found in Sarawak, Malaysia (*Calophyllum lanigrium*/ *Calophyllum lanigerium)*. (+)-Calanolide A is currently in early clinical trials in the United States [10].

Conospermum incurvum (saltbush) found in Western Australia and its chief constituent conocurovone have antitumor activity [8, 10].

Michellamine B obtained from the leaves of a vine found in the Korup rainforest region of southwest Cameroon (*Ancistrocladus korupensis)*, has undergone extensive preclinical study, but is considered too toxic for advancement to clinical test.

Synthetic derivative of *Dysoxylum binectiferum* compound are in early clinical trials for their antitumour activity [26].

*Agave americana (*Agavaceae) leaves contains steroidal saponins, alkaloid, coumarin, isoflavonoid, hecogenin and vitamins (A, B and C) have anticancerous activity. Plant is mainly found in Central America [27].

*Agrimonia pilosa (*Rosaceae) have anticancerous activity. It contains agrimonolide, flavonoid, triterpene, tannin and coumarin. Mainly found in China, Korea and Japan.

*Alpinia galanga (*Zinziberaceae) mainly found in Europe, its rhizome contains kaempferide and flavones [10].

Root of *Aster tataricus (*Asteraceae) contains triterpene, monoterpene and epifriedelanol. Generally found in Japan and Korea [10, 15].

*Chimaphila umbellate (*Ericaceae) contains ericolin, arbutin, urson and tannin. Plant is mainly found in Asia and Europe.

*Dryopteris crassirhizoma (*Polypodiaceae) rhizome is used for the treatment of tumor. It contains filicinic and filicic acids, aspidinol and aspidin. Plant is found in China, Japan and Korea [10].

*Erythronium americanum (*Liliaceae) contains alpha methylenebutyrolactone and have significant anticanceous activity. Mainly found in North America [10, 15].

*Eupatorium cannabinum (*Asteraceae) is mainly found in Europe and whole plant contains sesquiterpene, lactone, pyrrolizidine alkaloid [10, 15].

*Galium aparine (*Rubiaceae) plant contains iridoid, polyphenolic acid, tannin, anthraquinone. Galium plants are inhabitant of Europe, Africa and Australia [10, 15].

*Hydrastis canadensis (*Ranunculaceae) contains isoquinoline alkaloids (hydrastine, berberine, berberastine, candaline), resin and lactone. Mainly found in Canada and United states. This plant has been evaluated for their anticancerous activity [10, 15].

*Hypoxis argentea (*Hypoxidaceae**)** have antitumour activity. Mainly found in South Africa [15].

*Junchus effuses (*Juncaceae) contains tridecanone, effusol, juncanol and have antitumour activity. Plant is mainly found in China, Japan and Korea.

*Lantana camara (*Verbenaceae) it contains alkaloids (camerine, isocamerine, micranine, lantanine, lantadene), mainly found in Central America [10].
*Larrea tridentate (*Zygophyllaceae) contains resin. Plant is mainly found in Southwestern USA and Mexico and have anticancerous activity [28].
*Lonicera japonica (*Caprifoliaceae) contains tannins, saponins and carotenoids. Mainly found in China and used as anticancerous agent.
*Merwilla plumbea (*Hyacinthaceae**)** plants are generally found in South Africa. Bulb obtained from the plant is used for its anticancerous activity.
Root of *Panax quinquefolium (*Araliaceae**)** contains ginsenoside, sesquiterpene, limonene and vitamins (B1, B2, B12), panex plants are main inhibitants of China, Japan, Korea.
*Patrinia heterophylla (*Valerianaceae)- mainly found in China and have anticancerous activity.
Patriniaits scabiosaefolia (Valerianaceae) plant is generally found in China, Japan and Korea and used as anticancerous agent.
*Polygonatum multiflorum (*Liliaceae) plant contains saponin, flavonoid and vitamin A. Plant is mainly found in Asia or Europe and known for their anticancerous activity [10].
Bark *of Pygeum africanum (*Boraginaceae) contains phytosterol, triterpene and tannins. It is mainly found in Africa [10].
*Rhus chinensis (*Anacardiaceae) leaf contains tannin, apigenin and glycoside; seed contains bruceosides (A, B), brucein D and fatty oil. Plant is mainly found in China, Japan and Korea [10].
*Rubia akane (*Rubiaceae) contains anthraquinone and triterpene. Plant is mainly found in Japan and Korea. Extract of plant known for their antitumour activity [10].
*Rosmarinus officinalis (*Lamiaceae) contains volatile oil, borneal, carnosol, ursolic acid, diterpene. Plant is mainly found in South Europe and have antitnumour activity.
Leaves of plant Knowltonia capensis (Ranunculacea) have anticancerous activity. Plant is mainly found in South Africa [10, 15].
Ailanthus altissima (Simaroubaceae) is commonly known as “Tree-of-heaven”. According to Hartwell (1967–1971), the tree is used in homeopathic "remedies" for cancer. It is native to China and Taiwan [29].
Betula species (Betulaceae) contains betulinic acid, a pentacyclic triterpene, is a common secondary metabolite of plants used as anticancerous agent [30].
*Picrorrhiza kurroa (*Kutkin*)* **c**onsist of dried rhizome of plant *Picrorrhiza kurroa (*Scrophulariaceae*)* cut in small species and freed from attached rootlet. Drug is found to contain irridoid bitter substances, picrosides I, II and III and kutkoside. Picrosides and kutkoside monoterpine glycosides with an epoxy oxide in ring, has shown effective in liver cancer [32].
A flavonoid compound obtained from ripe seed of milk thistle Silybium marianum belonging to family Asteraceae (Compositae). It is effective in non melanoma skin cancer. Silymarine obtained from ripe seed composed

of flavolignine called Silydianin, silycrystine, silybin. Treatment with silybum has reduced tumor incidence from 100% to 60% ($P<0.003$) and tumor multiplicity by 78% ($P<0.0001$), with reduction in tumor volume per mouse by 90% ($P<0.003$) [33].

A polysaccharide from *Astralagus hedysarum* has a remarkable anti tumor activity. An intraperitoneal injection of 500mg/kg causes increase in the deposition of the third component of complement (C3) of macrophages in ICR mouse after two hours. When injection of *Astralagus hedysarum* was administered five times, the proportion of C3 positive macrophages was found to be more than that of one time injection. It suggests that *Astralagus hedysarum* has immuno-potentiating action [34].

A glycoside called Calotropine present in *Calotropis procera* (aak) exihibit anti tumor activity against human epidermoid carcinoma cells of rhinopharynx in vivo studies [35].

Hypericum perforatum (Hypericin) is commonly known as St. John's Wort. Hypericin belong to group of compound known as napthodianthrones. It has glioblastoma-inhibitory activity comparable to or greater than tamoxifen [36].

Herbal medicine today

The long tradition of herbal medicine continues to the present day in Asian countries like China, India, and many countries in Africa and South American. China is the country known for its traditional herbal medicine into a modern health care system. In a recent survey it was found that almost 7300 plants have been used in traditional Chinese medicine. Chinese apothecaries contain a number of dried plant specimens, and prescriptions are filled, not with prepackaged pills or ointments, but with measured amounts of specific herbs. Herbal medicines now days are the strong alternative for synthetic medicine as for as cost of development of a drug product is concern. Patients confronting a diagnosis of advanced (stage 4) cancer face the statistical reality that conventional chemotherapy can affect a cure only between 1-3% depending on the malignancy cases. The emerging integrative model of cancer treatment recognizes the importance of botanical medicine. Presently, research on anticancer drug development is largely dependent on exploring potential phytochemicals. Indian sub-continent is rich in its diversity of flora, being a tropical country with a large spread of rain forests and river basins. It is floristically rich with about 33 percent of its botanical wealth (over 15,000 species of higher plants) being endemic [31, 36].

References

[1] Samuelsson G. Drugs of natural origin: a Textbook of pharmacognosy, 4th edition, Stockholm, Swedish Pharmaceutical Press, 1999: 20-24

[2] Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod 1997; 1: 52-60.

[3]. Kappor LD. CRC Handbook of ayurvedic medicinal plants. Boca Raton, Florida, CRC Press, 1990: 416-417.

[4]. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Org 1985; 63: 965-981.

[5]. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

[6]. Dalziel TM.The Useful Plants of West Tropical Africa. 3rd ed. Bradford and London: Watmought Ltd, 1973: 526-530

[7]. Hartwell JL. Plants used against cancer: a survey. Lawrence, MA. Quarterman Publications, 1982: 438-439.

[8] Shoeb M, Celik S, Jaspars, M, Kumarasamy Y, MacManus S, Nahar L, Kong TLP, Sarker SD. Isolation, structure elucidation and bioactivity of schischkiniin, a unique indole alkaloid from the seeds of *Centaurea schischkinii*. Tetrahedron 2005; 61: 9001-9006.

[9] Jaspars M, Lawton LA. Cyanobacteria as a novel source of pharmaceuticals. Curr. Opinion Drug Discovery Dev. 1998; 1: 77-84.

[10] Cragg GM, Newman DJ. Plants as source of anticancer agents. J Ethnopharmacol 2005; 100: 72-79.

[11] Rowinsky EK, Onetto N, Canetta RM, Arbuck SG. Taxol-the 1st of the texanes: an important new class of anti-tumor agents. Semin Oncol 1992; 19: 646-662.

[12] Potmeisel M, Pinedo H. Camptothecins: new anticancer agents. Boca Raton, Florida, CRC Press 1995; 149-150.

[13] Bertino JR. Irinotecan for colorectal cancer. Semin Oncol 1997; 24: S18-S23.

[14] Itokawa H, Wang X, Lee K-H. Homoharringtonine and related compounds. In: Cragg GM, Kingston, DGI, New-man D, (eds). Anticancer agents from natural products. Boca Raton, Florida, Brunner-Routledge Psychology Press, Taylor & Francis Group 2005; 47-70.

[15] Powell RG, Weisleder D, Smith CR, Rohwedder WK. Structures of harringtonine, isoharringtonine, and homo-harringtonine. Tetrahedron Lett 1970; 11: 815-818.

[16] Dorai T, Cao YC, Dorai B. Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. Prostate 2001; 47: 293-303.

[17] Mehta RG, Moon RC.Characterization of effective chemopreventive agents in mammary gland *invitro* using an initiation-promotion protocol.Anticancer Res 1991; 11: 593-596.

[18] Soudamini NK, Kuttan R.Inhibition of chemical carcinogenesis by curcumin.J Ethnopharmacol 1989; 27: 227-233.

[19] Azuine M, Bhide S. Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr Cancer 1992; 17: 77-83.

[20] Xu Bin. Recent advances in pharmacologic study of natural anticancer agents in china. Mem. Ins. Oswaldo Cruz, Rio de janerio 1991; 86: 51-54.

[21] Trivedi N, Rawal UM.Effect of aqueous extract of *Andrographis paniculata* on liver tumour. Indian J Pharmacol 1998; 30: 318–322.

[22] Trivedi NP, Rawal UM. Hepatoprotective and antioxidant property of *Andrographis paniculata* in BHC induced liver damage in mice.Indian J Exp Biol 2001; 39: 41–46.

[23] Singh RP, Bannerjee S, Rao AR. Modulatory influence of *Andrographis paniculata* on mouse hepatic and extrahepatic carcinogen metabolising enzymes and antioxidant status. Phytotherapy Res 2001; 15: 382–390.

[24] Kapil A. Antihepatoxic effects of major diterpenoid constituents of *Andrographis paniculata*. Biochem Pharmacol 1993; 46: 182–185.

[25] Koul IB, Kapil A. Evaluation of the liver protective potential of piperine. Planta Med 1993; 59: 413–417.

[26] Christian MC, Pluda JM, Ho TC, Arbuck SG, Murgo AJ, Sausville EA. Promising new agents under development by division of cancer treatment, diagnosis, and centers of the National Cancer Institute. Semin Oncol 1997; 13: 2643-2655.

[27] Madhuri S, Pandey G.Some anticancer medicinal plants of foreign origin. Current Science 2009; 96(6): 780-781.

[28] Gonzales M, Bowden GT. Nordihydroguaiaretic acid-mediated inhibition of ultraviolet B-induced activator protein-1 activation in human keratinocytes. Molecular Carcinogenesis 2002; 34(2): 102-111.

[29] Hartwell, JL. Plants used against cancer,A survey. Lloydia. 1967–1971; 30–34.

[30] Cichewitz RH, Kouzi SA.Chemistry, biological activity, and chemotherapeutic potential of betulinic acid for the prevention and treatment of cancer and HIV infection. Med Res Rev. 2004; 24: 90-114.

[31] http://www.bioversityinternational.org/publications/Web_version/174/ch06.htm

[32] Jeena KJ, Joy KL, Kuttan R. Effect of *Emblica officinalis*, *Phyllanthus amarus* and *Picrorrhiza* [sic] *kurroa* on N-nitrosodiethylamine induced hepatocardinogenesis. Cancer Lett. 1999;136: 11–16.

[33] Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J Natl Cancer Inst 1997; 89(8): 556-566.

[34] Wang J, Ito H, Shimura K. Enhancing effect of antitumor polysaccharide from Astralagus or Radix hedysarum on C3 cleavage production of macrophages in mice. MemInst Oswaldo Cruz 1991; 86(2): 159-164.

[35] Willaman JJ, Hui Li L Lloydia. Alkaloid- bearing plants and their contained alka- loids 1970; 33(3A): 1.

[36] Alecu M, Ursaciuc C, Halalau F. Photodynamic treatment of basal cell carcinoma and squamous cell carcinoma with hypericin. Anticancer Res 1998;18: 4651-4654.