

Chapter 7

Biotechnology, In Vitro Production of Natural Bioactive Compounds, Herbal Preparation, and Disease Management (Treatment and Prevention)



Abstract Biotechnology uses living systems to develop products and plant biotechnology generates useful products or services, e.g., different bioactive secondary metabolites including alkaloids, flavonoids and other phenolics, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc., from plant cells, tissues or organs culture independent of geographical and climatic factors under aseptic conditions. These bioactive compounds are economically important as drugs (pharmaceuticals), flavors, perfumes (fragrances), pigments (dyes), agrochemicals as well as cosmetics, food additives, etc. Different strategies, e.g., genetic transformation of plants with *Agrobacterium rhizogenes*, hairy roots and others can be applied for the improvement of production of bioactive compounds of secondary metabolic origin. Recombinant DNA techniques can be used to manipulate metabolic pathways and produce protein pharmaceuticals such as antibodies, and protein hormones. Bioinformatics and genomics can find application in drug discovery from plant-based products and biotechnological procedures can enhance and advance the studies of medicinal plants. Molecular biotechnology uses laboratory techniques to study and modify nucleic acids and proteins for applications in areas such as human and animal health, agriculture, and the environment. Herbal extracts are now widely used in the management of chronic diseases like diabetes, hypertension, cancer, etc., as a part of CAM therapy. Plant-derived immune stimulators diverse small or large molecules (saponins, tomatine, inulin, polysaccharides), fungal β -glucans, complex molecules from marine sponge (α -galactosylceramide), shrimp chitin (chitosan), etc., have established adjuvant activity. Immunotherapy may be activation immunotherapy or suppression immunotherapy. Vaccines provide immune protection against diseases and plant-based edible vaccine production mainly involves the integration of transgene into the plant cells to produce the antigen protein for specific disease.

7.1 Biotechnology and Production of Bioactive Compounds and Techniques of Molecular Biotechnology

Biotechnology or biotech is the use of living systems and organisms to develop or make useful products or any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use, and plant biotechnology may be defined as generation of useful products or services from plant cells, tissues, and, often, organs (very small organ explants). Such cells, tissues, and organs are either continuously maintained *in vitro* or they pass through a variable phase to enable regeneration from them of complete plants which are ultimately transferred to the field.

Plants synthesize a large number of bioactive secondary metabolites including alkaloids, flavonoids and other phenolics, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc. The application of secondary bioactive compounds falls into five main categories, viz. drugs (pharmaceuticals), flavors, perfumes (fragrances), pigments (dyes), agrochemicals as well as cosmetics, food additives, etc. Most of these metabolites are obtained from plants as *in vitro* synthesis of these compounds at industrial level is difficult due to complex chemical structures and complicated biosynthetic pathways. Biotechnology offers a valuable tool to produce these compounds of interest in a desired amount and an eco-friendly way.

In vitro plant cell culture technique, in which plant cells, tissues, and organs are cultivated under aseptic conditions (independent of geographical and climatic factors), offers alternatives for producing important metabolites. The process includes mainly four main approaches, viz. callus, suspension, immobilized cells, and differentiated cultures. Callus culture involves growing a disorganized aggregate of cells from plant explants by culturing on a semi-solid support, which contains nutrients and any hormones required to promote growth of the cells. Suspension cultures result when callus is suspended in liquid growth medium and growing cells as dispersed cell culture. With their relatively fast growth, suspension cultures are widely employed in the study of bioactive secondary metabolite production by plant cells. The advantages of this approach are obvious, as biomass production is rapid than that of whole plant, nutritional and environmental requirements can be easily controlled allowing the production of pharmaceutical throughout the year if necessary, nutrient uptake is enhanced by submerged culture conditions, which stimulate the multiplication rate and higher yield of bioactive compounds. In several studies, *in vitro* culture were found more efficient than whole plant for the production of different bioactive secondary metabolites, i.e., the level of production of ajmalicine, ajmaline, anthraquinones, benzyloquinoline alkaloids, berberine, bisoclaurine, coniferin, diosgenin, ginseng, ginsenoside, glutathione, nicotine, rosmarinic acid, raucaffricine, shikonin, taxol, terpine, triptolide, ubiquinone-10, etc., were high *in vitro* culture compared to whole plant production following agronomic method

(Alamgir 2017). Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavors, fragrances, and colorants, which cannot be produced by microbial cells or chemical synthesis.

Plant tissue culture technology could be a potential alternative approach for bioproduction of phytoconstituents of therapeutic value and might be attractive under certain conditions if, for example, the source plant is difficult to cultivate, has a long cultivation period or has a low metabolite yield; if chemical synthesis has not been achieved or if it is technically problematic. In vitro culture of plant cell, tissue, and organs forms an integral part of any plant biotechnology activity. By employing biotechnological techniques, it is possible to regulate the biosynthetic pathway of plant in order to enhance/decrease the synthesis of particular compound. Different strategies can also be applied for the improvement of production of bioactive compounds of secondary metabolic origin. The major obstacles faced in the study of medicinal plants include inaccurate identification and speciation, low yield of bioactive metabolites, variability of traditional protocols, etc.

Research in the area of plant tissue and organ culture technology has resulted in the production of many bioactive metabolites (alkaloids, terpenoids, steroids and sterols, saponins, phenolics, flavonoids, amino acids, C-phycocyanins, glycosides, etc.) for new therapeutics under defined conditions. Bioactive compounds may be derived from microbial, algal, and vegetable sources and are used as antioxidants and anti-inflammatory agents, anti-allergenic compounds, etc. A recent development to overcome the difficulties arising with cell suspension cultures is the genetic transformation of plants with *Agrobacterium rhizogenes*. Hairy roots have been found to be suitable for the production of secondary metabolites, because of their stable and high productivity in hormone-free culture conditions. Recombinant DNA techniques can be used to manipulate metabolic pathways and produce protein pharmaceuticals such as antibodies, and protein hormones. The new disciplines of Bioinformatics and Genomics can find application in drug discovery from plant-based products and biotechnological procedures can enhance and advance the studies of medicinal plants. Plant-transformed technology has now reached a platform of commercial reality. Despite the advances in biotechnology techniques, there are only a few successful examples of secondary compounds production at an industrial level.

The biotechnological importance of secondary metabolites lies in the fact that: (i) many of them are commercially valuable chemicals, (ii) a number of them are toxic and should be removed from food products, and (iii) they act as protective agents, used by the plant against insects, pathogens, or animal foragers. The various objectives achieved/achievable by plant biotechnology may be summarized as follows: (i) Useful biochemical production (large-scale cell cultures); (ii) Rapid clonal multiplication (adventitious shoot/bulb/protocorm); (iii) Virus elimination (thermo-, cryo-, or chemotherapy coupled with meristem culture); (iv) Rapid development of homozygous lines by producing haploids (anther culture, ovary culture, interspecific hybridization); (v) Production/recovery of difficult to produce hybrids (embryo rescue, in vitro pollination); (vi) Germplasm conservation of

vegetatively reproducing plants or those producing recalcitrant seeds (cryopreservation, slow growth cultures, DNA clones); (vii) Genetic modification of plants (somaclonal variation, somatic hybridization, cybridization, and gene transfer); and (viii) Creation of genome maps and use of molecular markers to assist conventional breeding efforts.

Plants are the tremendous source for the synthesis of bioactive secondary metabolites, which are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives. Recent advances in the molecular biology, enzymology, and fermentation technology suggest that these plant products can be extracted from the aseptic culture of plant cell, tissue, and organ. Based on their biosynthetic origins, bioactive secondary metabolites of plant can be divided into three major groups such as (i) phenolic compounds; (ii) terpenoids; and (iii) nitrogen-containing alkaloids and sulfur-containing compounds. Compared to another group of secondary metabolites, phenolic compounds are largely responsible for beneficial effects on human health and these naturally occurring compounds are found largely in fruits, vegetables, cereals, and beverages. Phenolics that are not soluble are found in cell walls, while soluble phenolics are present within the plant cell vacuoles. Phenols are classified into different groups as a function of the number of phenol rings in the structure and their main classes include phenolic acids, flavonoids, stilbenes, and lignans and they include apigenin, diosmin, quercetin, kaempferol, eriodictyol, naringenin, hesperetin, baicalein, chrysin, catechin, morin, genistein, curcumin, colchicine, resveratrol, emodin, etc. Plant cell, tissue, and organ cultures have been established as routine work under sterile conditions from explants such as plant leaves, stems, roots, meristems, etc., for the production and extraction of hundreds of bioactive secondary metabolites (Karuppusamy 2009) (Table 7.1).

One of the most exciting aspects of cell culture technology is the potential for producing novel structures not observed in the parent plant, e.g., rutacultin by cultures of *Ruta graveolens* and sesquiterpene lactones by cultures of *Andrographis paniculata*. Table 7.2 shows some plant sources for different pharmaceutically valuable bioactive compounds.

The use of plant tissue cultures for the biotechnological production of bioactive phytoconstituents on commercial scale is attractive for several reasons. Tissue culture protocols have been developed for several plants but more are to be developed for many other species. Refined culture systems have improved the biochemical yields considerably, and over half a dozen cell cultures produce 2 g/l or more, of the biochemical.

7.1.1 Techniques of Molecular Biotechnology

Molecular biotechnology is the use of laboratory techniques to study and modify nucleic acids and proteins for applications in areas such as human and animal health, agriculture, and the environment. Molecular biotechnology results from the

Table 7.1 In vitro secondary metabolites from plant cell, tissue, and organs cultures

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
1. <i>Aconitum heterophyllum</i>	Aconites	MS + 2,4-D + Kinetin	Hairy root
2. <i>Adhatoda vasica</i>	Vasine	MS + BAP + IAA	Shoot culture
3. <i>Agastache rugosa</i>	Rosmarinic acid	MS + 2,4-D + Kinetin + 3% sucrose	Hairy root
4. <i>Agave amanuensis</i>	Saponins	MS + Kinetin	Callus
5. <i>Ailanthus altissima</i>	Alkaloids	MS + 2,4-D + Kinetin	Suspension
6. <i>Allium sativum</i>	Allin	MS + IAA + Kinetin	Callus
<i>Aloe saponaria</i>	Glucosides	MS + 2,4-D + Kinetin	Suspension
7. <i>Ambrosia tenuifolia</i>	Altamisine	MS + Kinetin	Callus
8. <i>Ammi majus</i>	Umbelliferone	MS + BAP	Shootlet
9. <i>Ammi visnaga</i>	Furanocoumarin	MS + IAA + GA3	Suspension
10. <i>Anchusa officinalis</i>	Rosmarinic acid	B5 + 2,4-D	Suspension
11. <i>Angelica gigas</i>	Deoursin	MS (Liq.) + 2,4-D + GA3	Hairy root
12. <i>Anisodus luridus</i>	Tropane alkaloids	MS + 2,4-D + BA	Hairy root
13. <i>Ammi majus</i>	Triterpenoid	MS + 2,4-D + BA	Suspension
14. <i>Arachys hypogaea</i>	Resveratrol	G5 + 2,4-D + Kin	Hairy root
15. <i>Armoracia laphthifolia</i>	Fusicoccin	MS + IAA	Hairy root
16. <i>Artemisia absinthum</i>	Essential oil	MS + NAA + BAP	Hairy root
17. <i>Artemisia annua</i>	Artemisinin	MS + IAA + Kinetin	Hairy root
18. <i>Artemisia annua</i>	Artemisinin	MS + NAA + Kinetin	Callus
19. <i>Aspidosperma ramiflorum</i>	Ramiflorin	MS + 2,4-D + BAP	Callus
20. <i>Aspidosperma ramiflorum</i>	Ramiflorin alkaloid	MS + 2,4-D + BAP + 30 g/l	Sucrose Callus
21. <i>Astragalus mongholicus</i>	Cycloartane saponin	MS + 2,4-D + Kin	Hairy root
22. <i>Astragalus mongholicus</i>	Cycloartane	MS + IAA + NAA	Hairy root
23. <i>Azadirachta indica</i>	Azadirachtin	MS + 2,4-D	Suspension
24. <i>Azadirachta indica</i>	Azadirachtin	MS + 2,4-D + Cyanobacterial elicitor	Suspension
25. <i>Beta vulgaris</i>	Betalain pigments	MS + IAA	Hairy root

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
26. <i>Brucea javanica</i>	Alkaloids	MS + 2,4-D + Kinetin	Suspension
27. <i>Brucea javanica</i>	Cathin	MS + IAA + GA3	Suspension
28. <i>Brugmansia candida</i>	Tropane	MS + 2,4-D + IAA	Hairy root
29. <i>Brugmansia candida</i>	Tropane alkaloid	MS + BA + NAA	Hairy root
30. <i>Bupleurum falcatum</i>	Saikosaponins	B5 + IBA	Root
31. <i>Bupleurum falcatum</i>	Saikosaponins	LS + 2,4-D	Callus
32. <i>Calystegia sepium</i>	Cuscohygrine	MS + 2,4-D + BA	Hairy root
33. <i>Camellia chinensis</i>	Flavones	MS + 2,4-D + NAA	Callus
34. <i>Camellia sinensis</i>	Theamine	MS + IBA + Kinetin	Suspension
35. <i>Campanula medium</i>	Polyacetylenes	MS + IAA + BA	Hairy root
36. <i>Canavalia ensiformis</i>	Canavanine	LS + NAA + Picloram	Callus
37. <i>Capsicum annum</i>	Capsiacin	MS + 2,4-D+ GA3	Callus
38. <i>Capsicum annum</i>	Capsiacin	MS + 2,4-D + Kin	Callus
39. <i>Capsicum annum</i>	Capsiacin	MS + 2,4-D + Kinetin	Suspension
40. <i>Cassia acutifolia</i>	Anthraquinones	MS + 2,4-D + kinetin	Suspension
41. <i>Cassia obtusifolia</i>	Anthraquinone	MS + TDZ + IAA	Hairy root
42. <i>Cassia senna</i>	Senosides	MS + NAA + Kin	Callus
43. <i>Catharanthus roseus</i>	Indole alkaloids	MS + IAA	Suspension
44. <i>Catharanthus roseus</i>	Indole alkaloids	MS + NAA + Kinetin	Suspension
45. <i>Catharanthus roseus</i>	Vincristine	MS + 2,4-D + GA3	Suspension
46. <i>Catharanthus roseus</i>	Indole alkaloid	MS + 2,4-D + GA3 + Vanadium	Suspension
47. <i>Catharanthus roseus</i>	Catharathine	MS + 2,4-D + UV-B radiation	Suspension
48. <i>Catharanthus trichophyllus</i>	Indole alkaloids	MS + IAA + GA3	Hairy root

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
49. <i>Cayratia trifoliata</i>	Stilbenes	MS + IAA + GA3	Suspension
50. <i>Centella asiatica</i>	Asiaticoside	MS + 2,4-D	Hairy root
51. <i>Centella asiatica</i>	Asiaticoside	MS + 2,4-D + Kin	Callus
52. <i>Centella asiatica</i>	Asiaticoside	MS + BAP + IAA	Shoot
53. <i>Centella asiatica</i>	Asiaticoside	MS + 2,4-D	Hairy root
54. <i>Centranthus ruber</i>	Valepotriates	MS + IAA + Kin	Hairy root
55. <i>Cephaelis ipecacuanha</i>	Alkaloids	MS + IAA	Root
56. <i>Chaenatis douglasei</i>	Thiarbrins	MS + NAA	Hairy root
57. <i>Chrysanthemum cinerariaefolium</i>	Pyrethrins	MS + 2,4-D + Kinetin	Callus
58. <i>Cinchona ledgeriana</i>	Quinine	MS + 2,4-D	Hairy root
59. <i>Cinchona succirubra</i>	Antraquinone	MS + IAA + GA3	Suspension
60. <i>Citrus</i> sp.	Limonin	MS + 2,4-D + Kinetin	Callus
61. <i>Coffea arabica</i>	Caffeine	MS + 2,4-D + Kinetin	Callus
62. <i>Coleus forskohlii</i>	Forskolin	MS + IAA + Kin	Hairy root
63. <i>Corydalis ambigua</i>	Corydaline	MS + IAA + 3% sucrose	Embryo
64. <i>Corydalis cava</i>	Corydaline	MS + IAA + GA3	Shoot
65. <i>Corydalis ophiocarpa</i>	Alkaloids	MS + 2,4-D + Kinetin	Callus
66. <i>Corydalis terminalis</i>	Corydaline	MS + 2,4-D + BAP	Callus
67. <i>Coscinium fenestratum</i>	Berberine	MS + 2,4-D + BAP	Callus
68. <i>Coscinium fenestratum</i>	Berberine	MS + IAA + BAP	Callus
69. <i>Coscinium fenestratum</i>	Berberine	MS + 2,4-D + GA3	Suspension
70. <i>Crataegus sinaica</i>	Flavonoid	MS + 2,4-D + NAA + BAP	Callus
71. <i>Croton sublyratus</i>	Plaunotol	MS + NAA + BA	Callus

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
72. <i>Cruciata glabra</i>	Antraquinones	LS + NAA + Kinetin	Suspension
73. <i>Cryptolepis buchanani</i>	Cryptosin	B5 + 2,4-D + Kinetin	Callus
74. <i>Cymbopogon citratus</i>	Essential oil	MS + IAA + GA3	Shoot
75. <i>Datura stramonium</i>	Hyocyanine	MS + IAA	Hairy root
76. <i>Digitalis purpurea</i>	Cardenolides	MS + BA	Suspension
77. <i>Digitalis purpurea</i>	Cardioactive glycosides	MS + 2,4-D + BA	Hairy root
78. <i>Diocorea doryophora</i>	Diogenin	MS + 2,4-D + BA	Suspension
79. <i>Dioscorea deltoidea</i>	Diosgenin	MS + 2,4-D	Suspension
80. <i>Drosera rotundifolia</i>	7-Methyljuglone	MS + BAP + NAA	Shoot culture
81. <i>Duboisia leichhardtii</i>	Alkaloids	LS + NAA + BA	Callus
82. <i>Duboisia leichhardtii</i>	Scopolamine	MS + 2,4-D + BA	Hairy root
83. <i>Duboisia myoporoides</i>	Scopolamine	MS + IAA	Hairy root
84. <i>Echinacea purpurea</i>	Alkamides	MS + 2,4-D	Hairy root
85. <i>Eleutherococcus senticosus</i>	Eleuthrosides	MS + 2,4-D	Suspension
86. <i>Ephedra</i> sp.	L-Ephedrine	MS + Kinetin + 2,4-D	Suspension
87. <i>Eriobotrya japonica</i>	Triterpenes	LS + NAA + BA	Callus
88. <i>Eucalyptus tereticornis</i>	Sterols and phenolic compounds	MS + 2,4-D	Callus
89. <i>Fabiana imbricata</i>	Rutin	MS + NAA + 2,4-D	Callus and Suspension
90. <i>Fagopyrum esculentum</i>	Flavonol	MS + IAA + GA3	Hairy root
91. <i>Fagopyrum esculentum</i>	Rutin	MS + NAA	Hairy root
92. <i>Frangula alnus</i>	Antraquinones	WPM + IAA + BAP	Callus
93. <i>Fritillaria unibracteata</i>	Alkaloids	MS + 2,4-D + Kin	Multiple shoot

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
94. <i>Fumaria capreolata</i>	Alkaloids	LS + IAA	Suspension
95. <i>Gentiana macrophylla</i>	Glucoside	MS + IAA + Kin	Hairy root
96. <i>Gentiana</i> sp.	Glucosides	B5 + Kinetin	Callus
97. <i>Gentianella austriaca</i>	Xanthone	MS + BAP	Multiple shoot
98. <i>Geranium thunbergii</i>	Tannin	MS + 2,4-D + BAP	Hairy root
99. <i>Ginkgo biloba</i>	Ginkoside-A	MS + NAA + Kinetin	Suspension
100. <i>Glehnia littoralis</i>	Furanocoumarin	LS + 2,4-D + Kinetin	Suspension
101. <i>Glycyrrhiza echinata</i>	Flavonoids	MS + IAA + Kinetin	Callus
102. <i>Glycyrrhiza glabra</i>	Triterpenes	MS + IAA + Kinetin + 2,4-D	Callus
103. <i>Glycyrrhiza glabra</i>	Glycyrrhizin	MS + 2,4-D + GA3	Hairy root
104. <i>Glycyrrhiza glabra</i>	Flavonoid	MS + IAA	Hairy root
105. <i>Gymnema sylvestre</i>	Gymnemic acid	MS + 2,4-D + IAA	Callus
106. <i>Gymnema sylvestre</i>	Gymnemic acid	MS + IAA + BA	Callus
107. <i>Gynostemma pentaphyllum</i>	Saponin	MS + 2,4-D + BAP	Hairy root
108. <i>Gypsophila paniculata</i>	Saponin	MS + IAA + TDZ	Root suspension
109. <i>Hemidesmus indicus</i>	Lupeol, Rutin	MS + BAP + NAA	Shoot culture
110. <i>Hyocyamus niger</i>	Tropane alkaloids	MS + 2,4-D + BA	Callus
111. <i>Hyocyamus niger</i>	Tropane alkaloids	MS + IAA + Kinetin	Hairy root
112. <i>Hyoscyamus albus</i>	Phytolexins	MS + NAA + GA3	Hairy root
113. <i>Hyoscyamus muticus</i>	Hyoscyamine	MS + 2,4-D	Hairy root
114. <i>Hypericum perforatum</i>	Hypericin liquid	MS + NAA + GA3	Suspension
115. <i>Hypericum perforatum</i>	Hypericins	MS + BA + IAA	Multiple shoot

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
116. <i>Hypericum perforatum</i>	Hypericin	MS + BA + TDZ	Multiple shoot
117. <i>Hypericum perforatum</i>	Hyperforin	MS + 2,4-D + Leusine	Multiple shoot
118. <i>Hyssopus officinalis</i>	Triterpenes	G5 + 2,4-D + IAA	Suspension
119. <i>Hyssopus officinalis</i>	Sterols	MS + 2,4-D + NAA	Suspension
120. <i>Ipomoea cairica</i>	Lignan	MS + IAA + Kin	Callus
121. <i>Isoplexis isabelliana</i>	Antraquinone	MS + 2,4-D + Kinetin	Suspension
122. <i>Lactuca virosa</i>	Sesquiterpene lactones	MS + 2,4-D	Hairy root
123. <i>Leontopodium alpinum</i>	Essential oil	MS + IAA + BA	Hairy root
124. <i>Linum flavum</i>	5-Methoxyphyllotaxin	MS salts + B5 Vitamins	Suspension
125. <i>Linum flavum</i>	Lignan	MS + IAA + GA3	Hairy root
126. <i>Lithospermum erythrorhizon</i>	Shikonin derivatives	LS + IAA + Kinetin	Suspension
127. <i>Lithospermum erythrorhizon</i>	Shikonin	MS + 2,4-D + Kinetin	Hairy root
128. <i>Lobelia cardinalis</i>	Polyacetylene glucosides	MS + 2,4-D	Hairy root
129. <i>Lycium chinense</i>	Cerebroside	MS + 2,4-D, Kinetin	Suspension
130. <i>Mentha arvensis</i>	Terpenoid	MS + BA + NAA	Shoot
131. <i>Momordica charantia</i>	Flavonoid	MS + BAP + NAA	Callus
132. <i>Morinda citrifolia</i>	Antraquinones	B5 + NAA	Suspension
133. <i>Mucuna pruriens</i>	L-Dopa	MS + IAA	Suspension
134. <i>Myristica fragrans</i>	Myristin	MS + NAA + TDZ	Shoot
135. <i>Nandina domestica</i>	Alkaloids	MS + 2,4-D + Kinetin	Callus
136. <i>Nicotiana glauca</i>	Anatabine	MS + IAA	Hairy root
137. <i>Nicotiana glauca</i>	Alkaloids	LS + 2,4-D + Kinetin	Callus

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
138. <i>Nicotianan tabacum</i>	Nicotine	MS + NAA + Kinetin	Suspension
139. <i>Ophiorrhiza rugosa</i> var. <i>decumbens</i>	Camptothecin	MS + BA + Kin	Shoot
140. <i>Panax ginseng</i>	Saponin and spogenins	MS + 2,4-D	Callus
141. <i>Panax ginseng</i>	Glycoside	MS + NAA + Kin	Hairy root
142. <i>Panax notoginseng</i>	Gensenosides	MS + 2,4-D + Kinetin	Suspension
143. <i>Papaver bracteatum</i>	Thebaine	MS + Kinetin + 2,4-D	Callus
144. <i>Papaver somniferum</i>	Alkaloids Morphine and codeine	MS + Kinein	Callus
145. <i>Papaver somniferum</i>	Codeine	LS + BA + NAA	Hairy root
146. <i>Peganum harmala</i>	Alkaloids	MS + 2,4-D	Suspension
147. <i>Perezia cuernavacana</i>	Sesquiterpene quinone	MS + IAA + BA	Hairy root
148. <i>Ophiorrhiza pumila</i>	Alkaloids	LS + 2,4-D + NAA	Callus
149. <i>Phytolacca americana</i>	Betacyanin	MS + 2,4-D	Suspension
150. <i>Picrasma quassioides</i>	Quassin	B5 + 2,4-D + Kinetin	Suspension
151. <i>Pimpinella anisum</i>	Essential oil	MS + IAA + BAP	Hairy root
152. <i>Piper solianum</i>	Piperine	MS + 2,4-D + BA	Suspension
153. <i>Plantago media</i>	Verbascoside	B5 + IAA + Kin	Callus
154. <i>Platycodon grandiflorum</i>	Polyacetylene	MS + 2,4-D	Hairy root
155. <i>Pluchea lanceolata</i>	Quercetin	MS + NAA + BAP	Callus
156. <i>Plumbago rosea</i>	Plumbagin	MS + CaCl ₂	Callus
157. <i>Plumbago zeylanica</i>	Plumbagin	MS + BAP + IBA	Hairy root
158. <i>Podophyllum hexandrum</i>	Podophyllotoxin	B5 + NAA	Suspension
159. <i>Podophyllum hexandrum</i>	Podophyllotaxin	MS + BAP + GA32	Shoot

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
160. <i>Polygala amarella</i>	Saponin	MS + IAA	Callus
161. <i>Polygonum hydropiper</i>	Flavonoids	MS + 2,4-D + Kinetin	Suspension
162. <i>Portulaca grandiflora</i>	Betacyanin	MS + 2,4-D + Kinetin	Callus
163. <i>Psoralea veris</i>	Saponins	MS + BAP + GA3	Shoot
164. <i>Psoralea corylifolia</i>	Isoflavones	MS + TDZ + BAP	Multiple shoot
165. <i>Ptelea trifoliata</i>	Alkaloids	MS + 2,4-D + Kinetin	Callus
166. <i>Rauvolfia sellowii</i>	Alkaloids	B5 + 2,4-D + Kinetin	Callus
167. <i>Rauvolfia serpentina</i>	Reserpine	LS + NAA + BA	Suspension
168. <i>Rauvolfia serpentina</i>	Serpentine	MS + BAP + IAA	Callus
169. <i>Rauvolfia serpentina</i>	Reserpine	MS + IAA + Cu ²⁺	Callus
170. <i>Rauvolfia tetraphylla</i>	Reserpine	MS + 2,4-D + Tryptophan	Callus
171. <i>Rhamnus catharticus</i>	Anthraquinones	WPM + Kin + 2,4-D	Callus
172. <i>Rheum ribes</i>	Catechin	MS + IBA + BA	Callus
173. <i>Rhodiola rosea</i>	Rosarin	MS + NAA + IAA	Callus culture
174. <i>Rhus javanica</i>	Gallotannins	LS + IAA + Kinetin	Root
175. <i>Rubia akane</i>	Anthraquinone	B5 + NAA + Kin	Hairy root
176. <i>Rubia akane</i>	Anthraquinone	MS + 2,4-D + Chitosan	Suspension
177. <i>Rubia tinctorum</i>	Anthraquinone	MS + 2,4-D	Hairy root
178. <i>Ruta</i> sp.	Alkaloids and coumarins	MS + 2,4-D + Kinetin	Callus
179. <i>Salvia miltiorrhiza</i>	Rosmarinic acid Cryptotanshinone	MS + 2,4-D + BA	Callus
180. <i>Salvia miltiorrhiza</i>	Rosmarinic acid Cryptotanshinone	MS + 2,4-D + Kinetin	Suspension
181. <i>Salvia officinalis</i>	Flavonoid	LMS + IAA + BAP	Multiple shoot
182. <i>Salvia officinalis</i>	Terpenoids	MS + 2,4-D + BA	Callus
183. <i>Saponaria officinalis</i>	Saponin	MS + IAA + TDZ	Suspension

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
184. <i>Saprosma fragrans</i>	Antraquinone	MS + 2,4-D + NAA	Callus
185. <i>Scoparia dulcis</i>	Scopadulic acid	LMS + Kin + Phenyl urea	Callus
186. <i>Scopolia parviflora</i>	Alkaloids	LS + 2,4-D + IAA	Callus
187. <i>Scutellaria baicalensis</i>	Flavonoids	MS + IAA	Hairy root
188. <i>Scutellaria columnae</i>	Phenolics	MS + 2,4-D + Kinetin	Callus
189. <i>Serratula tinctoria</i>	Ecdysteroid	MS + 2,4-D + BA	Hairy root
190. <i>Sesamum indicum</i>	Napthaquinone	MS + NAA + Kinetin	Hairy root
191. <i>Silybum marianum</i>	Silymarin	MS + IAA + GA3	Hairy root
192. <i>Silybum marianum</i>	Flavonolignan	LS + TDZ	Root
193. <i>Silybum marianum</i>	Silymarin	MS + IAA + Kin	Hairy root
194. <i>Silybum marianum</i>	Silymarin	MS + IAA + BA	Callus
195. <i>Simmondsia chinensis</i>	Fixed oil	MS + TDZ + GA3	Callus
196. <i>Simmondsia chinensis</i>	Fixed oil	MS + IAA + 2iP	Callus
197. <i>Solanum aculeatissi</i>	Steroidal saponin	MS + 2,4-D	Hairy root
198. <i>Solanum chrysotrichum</i>	Saponin	MS + 2,4-D + Kinetin	Suspension
199. <i>Solanum laciniatum</i>	Solasodine	MS + 2,4-D + Kinetin	Suspension
200. <i>Solanum paludosum</i>	Solamargine	MS + BA + Kinetin	Suspension
201. <i>Stevia rebaudiana</i>	Stevioside	MS + BA + NAA	Callus
202. <i>Swertia japonica</i>	Amarogenetin	MS + IAA	Hairy root
203. <i>Tabernaemontana divaricata</i>	Alkaloids	MS + NAA + BA	Suspension
204. <i>Tagetes patula</i>	Thiophenes	MS + IAA + Kinetin	Hairy root
205. <i>Tanacetum parthenium</i>	Sesquiterpene	MS + 2,4-D + Kinetin	Hairy root

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
206. <i>Taxus baccata</i>	Taxol baccatin III	B5 + 2,4-D + Kinetin + GA3	Suspension
207. <i>Taxus</i> spp.	Taxol	B5 + 2,4-D + BA	Suspension
208. <i>Thalictrum minus</i>	Berberin	LS + NAA + 2,4-D + BA	Suspension
209. <i>Tinospora cordifolia</i>	Berberin	MS + IAA + GA3	Suspension
210. <i>Torreya nucifera</i>	Diterpenoids	MS + 2,5-D	Suspension
211. <i>Trichosanthes kirilowii</i>	Protein	MS + IAA	Hairy root
212. <i>Trigonella foenum-graecum</i>	Saponins	MS + 2,4-D + Kinetin	Suspension
213. <i>Vaccinium myrtillus</i>	Flavonoids	MS + BAP + NAA	Callus culture
214. <i>Vinca major</i>	Vincamine	MS + BAP	Hairy root
215. <i>Vitis vinifera</i>	Anthocyanin	MS + BAP + NAA	Suspension
216. <i>Vitis vinifera</i>	Resveratrol	MS + IAA + GA3 + UV	Callus
217. <i>Withania somnifera</i>	Withaferin A	MS + BA	Shoot
218. <i>Withania somnifera</i>	Withaferin	MS + IAA + Kintin	Hairy root
219. <i>Withania somnifera</i>	Withanoloid A	MS + IAA + Kin	Hairy root
220. <i>Withania somnifera</i>	Steroidal lactone	MS + 2,4-D + BA	Callus
221. <i>Zataria multiflora</i>	Rosmarinic acid	MS + IAA + Kin	Callus

convergence of many areas of research, such as molecular biology, microbiology, biochemistry, immunology, genetics, and cell biology; and includes techniques such as molecular cloning, polymerase chain reaction, gel electrophoresis, macro-molecule blotting and probing, microarrays, allele-specific oligonucleotide, high throughput screening (HTS), techniques of in vitro synthesis of bioactive molecules, etc. It is an exciting field fueled by the ability to transfer genetic information between organisms with the goal of understanding important biological processes or creating a useful product. Information from human genomics project has opened a myriad of opportunities to create new medicines and treatments, as well as approaches to improve existing medicines. Molecular biotechnology is a rapidly changing and dynamic field. The importance and impact of molecular biotechnology is being felt across the nation. Molecular biotechnology has applications in

Table 7.2 Pharmaceutically valuable bioactive compounds obtained from different plant sources

Compound	Plant species	Medicinal value
(i) Shikonin	<i>Lithospermum erythrorhizon</i>	Antiseptic (also used as dye for silk and cosmetics)
(ii) Berberine	<i>Coptis japonica</i>	Antibacterial, anti inflammatory
(iii) codeine	<i>Papaver somniferum</i>	Analgesic
(iv) Diosgenin	<i>Dioscorea deltoidea</i>	Antifertility agents
(v) Quinine	<i>Cinchona</i>	Antimalarial
(vi) Scopolamine	<i>Datura stramonium</i>	Antihypertensive
(vii) Vincristine	<i>Catharanthus roseus</i>	Antileukemic
(viii) Ajmalicine	<i>C. roseus</i>	
(ix) Taxol ^a	<i>Taxus species</i>	Breast and ovarian cancer treatment
(x) Artemisin	<i>Artemisia</i> sp.	Antimalarial
(xi) Trichosanthin	<i>Trichosanthes</i> sp.	Cytotoxicity against HIV infected cells, immunosuppressant, induces abortion
(xii) Karasurin ^b		

^aActs on spindle-like colchicine; promotes dissolution of microtubules into tubulin molecules;

^bProteins isolated from rhizomes of the traditional Chinese medicinal plant

plant and animal agriculture, aquaculture, chemical and textile manufacturing, forestry, and food processing and the tools of molecular biotechnology can be applied to develop and improve drugs, vaccines, therapies, and diagnostic tests that will improve human and animal health.

7.2 Advantages of Tissue Cultures in Production of Useful Bioactive Compounds

In in vitro technique for plant bioactive compound production, plant cells, tissues and organs are cultivated under aseptic conditions independently of geographical and climatic factors. It offers an alternative approach for producing important bioactive metabolites in the face of different adverse circumstances such as circumstances loss of plant populations, genetic diversity, habitat degradation and, even, species extinction, etc. It has emerged as a viable biotechnological tool for the production of bioactive compounds that can be used in the most diversified areas and particularly with a view of an additional effort for sustainable conservation and rational utilization of biodiversity. Plant tissue culture technology could be a potential alternative approach for production of high-value bioactive phytoconstituents of therapeutic importance and might be attractive under certain conditions such as: when (i) the source plant is difficult to cultivate, (ii) has a long cultivation

period, (iii) has a low metabolite yield, (iv) chemical synthesis has not been achieved due to technical problem, etc. In addition, (v) novel compounds which are not generally found in the parent plants can be produced in the in vitro grown plants through plant tissue culture as well as (vi) stereo- and region-specific biotransformation of the plant cells can be performed for the production of bioactive compounds from economical precursors.

With the increasing demand of the market for novel products derived from plants, in vitro culture has become a reliable technique for the mass production of plant material. These and a number of other advantages in using plant cell culture provide impetus for its use for large-scale production of important bioactive compounds at industrial level. These advantages are summarized as follows:

- (i) Plant cell cultures are independent from environmental factors;
- (ii) Production levels may be geared more accurately according to the market demand;
- (iii) By using characterized cell lines, a more consistent product quality and yield can be maintained;
- (iv) New routes of synthesis can be recovered from mutant cell lines which may lead to the development of novel products;
- (v) Culture of cells will reduce the pressure on already overexploited medicinal and other economically important plants.
- (vi) The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products;
- (vii) The advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions;
- (viii) The use of in vitro plant cell culture for the production of chemicals and pharmaceuticals has made great strides building on advances in plant science;
- (ix) The increased use of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolism will provide the basis for the production of commercially acceptable levels of product;
- (x) The increased level of natural products for medicinal purposes coupled with the low product yields and supply concerns of plant harvest has renewed interest in large-scale plant cell culture technology;
- (xi) Knowledge of biosynthetic pathways of desired phytochemicals in plants as well as in cultures is often still in its infancy, and consequently strategies needed to develop an information based on a cellular and molecular level. These results show that in vitro plant cell cultures have potential for commercial production of secondary metabolites; and
- (xii) The introduction of newer techniques of molecular biology, so as to produce transgenic cultures and to effect the expression and regulation of biosynthetic pathways, is also likely to be a significant step toward making cell cultures more generally applicable to the commercial production of secondary metabolites.

7.3 Herbal Preparations and Disease Management (Prevention and Treatment)

Medicinal plants play an important role in preventing and treating of human diseases. The bioactive phytochemicals present in them play important role in health promotion and disease prevention. The curative efficacy of medicinal plants is due to these bioactive metabolites (pharmacologically active compounds), e.g., alkaloids have an antispasmodic, antimalarial, analgesic, diuretic activities; phenols and flavonoids have an antioxidant, anti-allergenic, antibacterial properties; terpenoids are known for their antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory properties; glycosides are reported for antifungal and antibacterial properties; and saponins are reported to have anti-inflammatory, antiviral, plant defense activities. The use of medicinal herbs and herbal preparation is an age-old tradition and the recent progress in modern therapeutics has stimulated the use of these natural therapeutics in the prevention and treatment of different diseases. Different bioactive compounds from different plant sources are found to be effective against a vast array of diseases, e.g., taxol from *Taxus brevifolia* and vinblastine and vincristine from *Catharanthus roseus*, topotecan and irinotecan from *Camptotheca acuminata*, etoposide and teniposide from *Podophyllum peltatum* show antitumor and anticancer activity; curcumin from *Curcuma longa* show anticancer, anti-inflammatory, hepatoprotective; flavonoid silymarin (silibinin) from *Silybum marianum* show anticancer, anti-inflammatory, liver tonic for hepatic disorders property; ricinine), lectin (ricin) from *ricinus communis* show hepatoprotective, antioxidant, hypoglycemic, antitumor activity; tannins, shikimic acid compounds, triterpenoids, ellagic acid from *Terminalia chebula* show antioxidant, antidiabetic, renoprotective, hepatoprotective activity; steroidal lactones, withanolides, notably withaferin A from *Withania somnifera* show chemopreventive, anticancerous, memory enhancer, and immunomodulatory properties and used in parkinson's and alzheimer's disorders; mono and sesquiterpenoids, zingerone and gingerols from *Zinziber officinalis* are anticancer, antioxidant, hepatoprotective, hypercholesterolaemic, anti-atherosclerotic; limonoids (nimbidiin), di- and tri- terpenoids from *Azadirachta indica* function as inhibitor of carcinoma, chemopreventive, inhibit colon cancer, antiallergic, blood purifier; piperidine, dehydropiperonaline of *Piper nigrum* are anticarcinogenic, anti-hyperlipidaemic, useful in epilepsy; diterpenoid furanolactones (tinosporin), isoquinoline alkaloids from *Tinospora cordifolia* function as immunomodulator, chemopreventive, cardioprotective, antidiabetic agents; aloin and emodin, campesterol, β -sisosterol from *Aloe vera* show healing properties, antiviral and antitumor activity antidiabetic, hepatoprotective, antiseptic effect; apigenin, taxol and ursolic acid, citral from *Ocimum sanctum* show antidiabetic, hepatoprotective antibacterial, antifungal, antipyretic, and anticancer properties; berberine from *Berberis vulgaris* works as antidiabetic, hepatoprotective, antimicrobial; digoxin, from *Digitalis lanata* is used in heart diseases; thymoquinone from *Nigella sativa* show antidiabetic, anticancer, antimicrobial, hepatorenal protective, and gastro-protective; quinq quinine from *Cinchona robusta*

show antimalarial, antiparasitic effect; artemisinin from *Artemisia absinthium* is an antimalarial drug; ophelic acid, sawertiamarine, mangleferin and amarogenitine from *Swertia chirata* show antidiabetic antiviral, hepatorenal protective activities; allicin from *Allium sativum* is cardioprotective, anti-inflammatory; arjunic acid, tannic acid, tannins, saponins, gallic acid and phytosterols from *Terminalia arjuna* are cardioprotective, anticancer agents, hepatoprotective; emblicanin A, emblicanin B, punigluconin, and pedunculagin from *phyllanthus emblica* are antiviral, antimicrobial, anticancer, hepatoprotective and antidiabetic; ajmalicine and reserpine from *Rauvolfia serpentina* show hypotensive properties, phenol compounds from *Gynura procumbens* are antidiabetic, etc.

7.3.1 Herbal Extracts and Management of Chronic Diseases

Herbal medicines are the most preferred ways of complementary and alternative medicine (CAM used all over the world for the management of different chronic diseases. Diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), etc., among others, are the most prevalent chronic diseases in the world. Some other leading chronic diseases include osteoporosis, cardiovascular disease (heart attacks, stroke, ischemic cardiopathy), chronic obstructive pulmonary disease (COPD), asthma, epilepsy and seizures, obesity, oral health problems, hepatitis C and HIV/AIDS, dementia, Alzheimer's, autoimmune, and Parkinson's disease, schizophrenia, bipolar disorder, multiple sclerosis, glaucoma, etc. Patients with chronic diseases frequently or occasionally use herbal medicines including CAM all over the world for treatment because of diversity, low cost, and safety.

In a work with a total of 217 patients (55 male and 162 female and 56.6 ± 9.7 years mean age of the participants), Tulunay et al. (2015) noted about 29% patient used herbal medicine use and use among female gender was significantly higher ($P = 0.040$). Conventional medication use was found to be lower among herbal medicine consumers and the most frequently used herbs were lemon (39.6%) and garlic (11.1%) for HT, cinnamon (12.7%) for DM, and walnut (6.3%) for HL. A number of photochemicals (nutraceuticals), e.g., omega-3-fatty acids, dietary fibers, vitamins, antioxidants, plant sterols, flavonoids from the medicinal plants that have beneficial effects on the chronic diseases and nutraceutical photochemicals without any side effects, less cost and also abundant helps to prevent a number of chronic diseases and act as chronic fighters.

Diabetes management

Diabetes mellitus (DM) should not be confused with diabetes insipidus (DI). Diabetes insipidus occurs when a person's kidneys pass an abnormally large volume of urine that is insipid (dilute and odorless). In most people, the kidneys pass about 1–2 quarts of urine a day, but in person with DI, the kidneys can pass 3–20 quarts of urine a day. As a result, a person with DI may feel the need to drink large

amounts of liquids. DM results from insulin deficiency or resistance leading to high blood glucose, also called blood sugar. Diabetes insipidus and diabetes mellitus are unrelated, although both conditions cause frequent urination and constant thirst. Diabetes mellitus causes high blood glucose, or blood sugar, resulting from the body's inability to use blood glucose for energy. People with diabetes insipidus have normal blood glucose levels; however, their kidneys cannot balance fluid in the body. Excessive urination and extreme thirst as a result of inadequate output of the pituitary hormone ADH (antidiuretic hormone—vasopressin) (Fig. 7.1) or the lack of the normal response by the kidney to ADH. There are four types of diabetes insipidus; (i) central diabetes insipidus, (ii) nephrogenic diabetes insipidus, (iii) dipsogenic diabetes insipidus, and (iv) gestational diabetes insipidus. The most common symptom of DI is frequent urination and it is a rare disorder, i.e., a rare disease that causes frequent urination.

Diabetes mellitus (DM) is one of the most common and serious chronic diseases worldwide. DM is a chronic endocrine disorder involving most common metabolic disorders of carbohydrate, fat, and protein which are grouped under non-communicable disease (NCD). DM is characterized by elevated plasma glucose concentrations resulting from insufficient insulin. Type 1 diabetes is called insulin-dependent diabetes mellitus (IDDM) or juvenile-on set diabetes and type 2 diabetes is called noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. The National Institute for Clinical Excellence (NICE) recommended target blood glucose level ranges for normal and diabetic blood sugar. For the majority of healthy individuals, normal blood sugar levels are as follows (NICE:Diabetes.co.uk: https://www.diabetes.co.uk/diabetes_care/blood-sugar-level-ranges).

(i) Between 4.0 and 6.0 mmol/L (72–108 mg/dL) when fasting; (ii) Up to 7.8 mmol/L (140 mg/dL) 2 h after eating. For people with diabetes, blood sugar level targets are as follows; (iii) Before meals: 4–7 mmol/L for people with type 1 or type 2 diabetes; (iv) After meals: under 9 mmol/L for people with type 1 diabetes and under 8.5 mmol/L for people with type 2 diabetes. Table 7.3 summarizes these data.

Fig. 7.1 Vasopressin, an antidiuretic hormone (ADH) also arginine vasopressin (AVP) or argipressin. It is synthesized as a peptide prohormone in neurons in the hypothalamus, and then converted to AVP

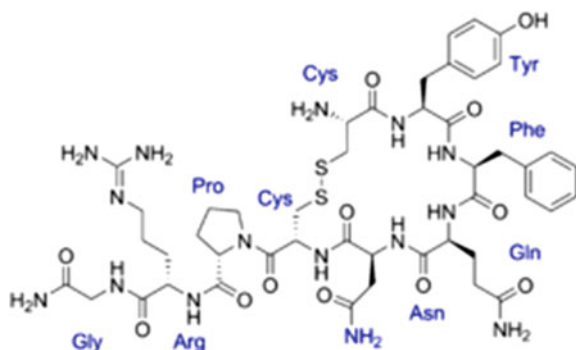


Table 7.3 NICE recommended target blood glucose level ranges

Target levels by type	Upon waking	Before meals (pre-prandial)	At least 90 min after meals (postprandial)
Nondiabetic ^a		4.0–5.9 mmol/L	under 7.8 mmol/L
Type 2 diabetes		4–7 mmol/L	under 8.5 mmol/L
Type 1 diabetes	5–7 mmol/L	4–7 mmol/L	5–9 mmol/L
Children w/type 1 diabetes	4–7 mmol/L	4–7 mmol/L	5–9 mmol/L

^aThe non-diabetic figures are provided for information but are not part of NICE guidelines

Table 7.4 Blood sugar levels in diagnosing diabetes

Blood sugar levels in diagnosing diabetes			
Plasma glucose test	Normal	Prediabetes	Diabetes
Random	Below 11.1 mmol/L below 200 mg/dL	N/A	11.1 mmol/L or more 200 mg/dL or more
Fasting	Below 6.1 mmol/L below 108 mg/dL	6.1–6.9 mmol/L to 108–125 mg/dL	7.0 mmol/L or more 126 mg/dL or more
2 h postprandial	Below 7.8 mmol/L below 140 mg/dL	7.8–11.0 mmol/L to 140–199 mg/dL	11.1 mmol/L or more 200 mg/dL or more

The data on the range of blood sugar levels in diagnosing diabetes and prediabetes and prediabetes are shown in the Table 7.4.

Random plasma glucose test: A blood sample for a random plasma glucose test can be taken at any time. This does not require much planning and is, therefore, used in the diagnosis of type 1 diabetes when time is of the essence.

Fasting plasma glucose test: A fasting plasma glucose test is taken after at least 8 h of fasting and is, therefore, usually taken in the morning. The NICE guidelines regard a fasting plasma glucose result of 6.1–6.9 mmol/L as putting someone at higher risk of developing type 2 diabetes, particularly when accompanied by other risk factors for type 2 diabetes.

Oral glucose tolerance test (OGTT): An oral glucose tolerance test involves first taking a fasting sample of blood and then taking a very sweet drink containing 75 g of glucose. After having this drink, you need to stay at rest until a further blood sample is taken after 2 h.

HbA1c test for diabetes diagnosis: An HbA1c test does not directly measure the level of blood glucose, however, the result of the test is influenced by how high or low your blood glucose levels have tended to be over a period of 2–3 months.

Indications of diabetes or prediabetes are given under the following conditions:

- (i) **Normal:** Below 42 mmol/mol (6.0%)
- (ii) **Prediabetes:** 42–47 mmol/mol (6.0–6.4%)
- (iii) **Diabetes:** 48 mmol/mol (6.5% or over)

A comprehensive herbal drug therapeutic regimen offers time-tested safe and effective support to conventional therapy in the management of diabetes. This is combination with adequate dietary management and physical activity would provide an integrated approach to the management of this deadly disease, particularly Type 2 diabetes. Development of type 2 diabetes has been linked to β -cell failure coupled with *insulin* resistance and obesity. Adipose tissue, known as the fat store, secretes a number of hormones and proteins collectively termed adipokines some of which regulate *insulin* sensitivity.

Diabetes is a metabolic, endocrine disorder which is characterized by hyperglycemia and glucose intolerance due to insulin resistance and researchers have confirmed that inflammation is closely involved in the pathogenesis of diabetes and its complications. Herbal medications have long been used in the treatment and prevention of T2DM in both traditional Chinese medicine (TCM) and traditional Indian medicine (Ayurveda, Unani). Existing therapeutic drugs used for diabetes increase various secondary complications including cardiovascular disease, kidney failure, liver injury, dizziness, mental disorders, weight gain, and skin diseases. Nowadays, the key therapeutic agents to treat T2DM and its complications, sulfonylureas, metformin, and insulin-sensitizing glitazones all improve metabolic control and lead to control of various circulating inflammation mediators through innate immunity-related signaling pathways. Sulfonylureas and metformin are main drugs to prevent the T2DM, and sulfonylureas increase insulin production from pancreatic β -cells, while metformin suppresses glucose production in the liver and meanwhile increases insulin sensitivity in peripheral tissues. Glitazones, another antidiabetic drug, binds to peroxisome proliferator-activated receptors (PPARs), beginning a transcriptional activity that leads to improved insulin action through reducing the secretion of inflammatory markers. Consequently, glitazones reduced levels of CRP, PAI-1, TNF- α , and other inflammatory markers. These drugs showed better antidiabetic nature and also have the comparable anti-inflammatory potential.

Other therapeutic approaches for T2DM that would act as principals in the inflammatory system have been proposed in the form of salicylates, an anti-inflammatory therapeutic that inhibits κ B kinase (IKK), and also lowering the glucose level through improvement of beta cell function. Various well-established nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase inhibitors (e.g., ibuprofen, naproxen) are able to improve glucose-mediated macrosomia. Researchers are searching for efficient natural therapeutic targets with less or no side effects from natural products'-derived bioactive molecules to improve insulin resistance and associated complications through suppression of inflammatory signaling pathways.

Table 7.5 Some selected botanical therapeutics and their proposed mode of carbohydrate metabolism

Botanical therapeutics	
Scientific name	Proposed actions
<i>Hoodia gordonii</i>	WL, decreases appetite
<i>Opuntia</i> spp.	Decreases LDL; decreases TG; decreases PPG; decreases IS
<i>Cinnamomum cassia</i> , <i>Cinnamomum verum</i> , and others	Increases IS; decreases FPG; decreases PPG; decreases BP; decreases LDL; decreases TG
<i>Artemisia dracuncululus</i> L.	Increases IS; decreases PPG
<i>Momordica charantia</i>	Increases IS; decreases FPG; decreases PPG; decreases LDL; decreases TG
<i>Trigonella foenum-graecum</i>	Increases IS; decreases FPG; decreases LDL; decreases TG
<i>Gymnema sylvestre</i>	Increases IS; decreases FPG; decreases PPG; decreases LDL; decreases TG; increases Ins sec
<i>Allium sativum</i>	Decreases BP; decreases LDL
<i>Ginkgo biloba</i>	Decreases BP
<i>Panax</i> spp.	Decreases BP
<i>Aloe vera</i>	Increases IS; decreases FPG
<i>Coccinia indica</i>	Increases IS; decreases FPG

Source Cefalu et al. (2011)

BP = blood pressure; LDL = LDL-cholesterol; TG = triglycerides; FPG = fasting blood glucose; PPG = postprandial blood glucose; IS = insulin sensitivity; WL = weight loss; and Ins sec = insulin secretion.

There are a huge number of active medicinal plants and its natural bioactive molecules that have already reported the therapeutic nature against diabetes. Several medicinal plants have been used since ancient times to manage and prevent diabetes and associated conditions (Table 7.5).

All parts of antidiabetic plants are not equally important for the preparation of hypoglycemic extract, so different parts (including the whole plant to leaf, root, rhizome, flower, fruit, seed, etc.) of antidiabetic plants are used for their desired hypoglycemic activity as shown in Table 7.6.

Mode of action of different bioactive phytoconstituents in hyperglycemia

Different bioactive phytoconstituents follow similar or different ways to alleviate hyperglycemic activity as

- (i) Alkaloids—Inhibit alpha glucosidase and decrease glucose transport through the intestinal epithelium, e.g., berberine (roots, stem bark of *Berberis* spp., *T. cordifolia*); casuarine 6-*O*- α -glucoside (bark of *Syzygium malaccense*); catharanthine, vindoline, and vindoline (leaf and stem of

Table 7.6 Some selected antidiabetic plants with hypoglycemic activity distributed in their whole body or in different parts

Sl. no.	Plant/part	Name of plants
(i)	Whole plant	<i>Abies pindrow</i> , <i>Achyranthes aspera</i> , <i>Ajuga iva</i> , <i>Aloe vera</i> , <i>Anacardium occidentale</i> , <i>Andrographis paniculata</i> , <i>Capsicum frutescens</i> , <i>Cryptolepis sanguinolenta</i> , <i>Enicostemma littorale</i> , <i>Ficus religiosa</i>
(ii)	Roots	<i>Clausena anisata</i> , <i>Glycyrrhiza glabra</i> , <i>Helicteres isora</i> , <i>Pandanus odoros</i>
(iii)	Rhizome	<i>Nelumbo nucifera</i>
(iv)	Bulb	<i>Allium cepa</i> , <i>Allium sativum</i>
(v)	Modified root	<i>Ipomoea batata</i>
(vi)	Aerial parts	<i>Artemisia pallens</i> , <i>Bidens pilosa</i> , <i>Bixa orellana</i> , <i>Teramnus labialis</i>
(vii)	Leaves	<i>Aloe barbadensis</i> , <i>Annona squamosa</i> , <i>Averrhoa bilimbi</i> , <i>Azadirachta indica</i> , <i>Beta vulgaris</i> , <i>Camellia sinensis</i> , <i>Cassia alata</i> , <i>Eclipta alba</i> , <i>Eucalyptus globulus</i> , <i>Euphrasia officinale</i> , <i>Ficus carica</i> , <i>Gymnema sylvestre</i> , <i>Gynura procumbens</i> , <i>Ipomoea aquatica</i> , <i>Mangifera indica</i> , <i>Myrtus communis</i> , <i>Memecylon umbellatum</i> , <i>Morus indica</i> , <i>Ocimum sanctum</i> , etc.
(viii)	Flower	<i>Cassia auriculata</i> , <i>Gentiana olivier</i> , <i>Musa sapientum</i>
(ix)	Fruit	<i>Carum carvi</i> , <i>Coriandrum sativum</i> , <i>Embellica officinalis</i> , <i>Juniperus communis</i> , <i>Momordica charantia</i> , <i>Xanthium strumarium</i>
(x)	Seed	<i>Acacia arabica</i> , <i>Agrimony eupatoria</i> , <i>Lupinus albus</i> , <i>Luffa aegyptiaca</i> , <i>Lepidium sativum</i> , <i>Mucuna pruriens</i> , <i>Punica granatum</i>
(xi)	Stem	<i>Amaranthus spinosus</i> , <i>Coscinium fenestratum</i>
(xii)	Bark	<i>Cinnamomum zeylanicum</i> , <i>Croton cajucara</i>

C. roseus); calystegine B2 (fruits of *Nicandra physalodes*); cryptolepine (*Cryptolepis sanguinolenta*); harmane, norharmane, (*Tribulus terrestris*); jambosine (bark, seeds, fruits, *Syzygium cumini*); jatrorrhizine, magnoflorine, palmatine (*T. cordifolia*); javaberine A, javaberine A hexaacetate, javaberine B hexaacetate (roots of *Talinum paniculatum*); lepidine and semilepidine (seeds of *Lepidium sativum*); lupanine (*Lupinus perennis*); mahanimbine (leaf of *Murraya koenigii*); piperumbellactam A (branches of *Piper umbellatum*); radicamines A and B (*Lobelia chinensis*); swerchirin (*S. chirayita*); tecomine (*Tecoma stans*); trigonelline (seeds of *Trigonella foenum-graecum*; 1-deoxynojirimycin (leaf and bark of *Morus alba*);

- (ii) Imidazoline compounds—Stimulates insulin secretion in glucose-dependent manner; they are insulinotropic, e.g., the imidazoline RX871024 was found to increased basal- and glucose-stimulated insulin release in vitro and in vivo;
- (iii) Polysaccharides—Increased the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose. polysaccharides like

- aconitans A-D (roots of *Aconitum carmichaeli*); atractans A (rhizome of *Atractylodes japonica*); ganoderans A and B (fruit bodies of *Ganoderma lucidum*); galactomannan gum (seeds and tubers of *Cyamopsis tetragonolobus* and *Amorphophallus konjac*);
- (iv) Flavonoids—Suppressed the glucose level, reduced plasma cholesterol and triglycerides significantly, and increased their hepatic glucokinase activity probably. Bengalenoside flavonoids (stem bark of *Ficus benghalensis*); cyanidin-3-galactoside; epigallocatechin gallate (leaf of *Camellia sinensis*); (-)-3-*O*-galloylprocatechin, (-)-3-*O*-galloylcatechin (*Bergenia ciliata*); genistein (*Glycine* spp. and soya beans); hesperidin, naringin (*Citrus* spp.); prunin (stem of *Amygdalus davidiana* var. *davidiana*); kaempferitrin (leaf of *Bauhinia forficata*); kaempferol (leaf of *Jindai*, Soybean); kolaviron (*Garcinia kola*); leucodelphinidin (bark of *Ficus benghalensis*); mangiferin (rhizome of *Anemarrhena asphodeloides*); marsupsin, pterostilbene (heartwood of *Pterocarpus marsupium*); quercetin (*Chamaecostus cuspidatus*); Rutin; shamimin (leaf of *Bombax ceiba*); leaves
- (v) Dietary fibers—Effectively adsorbed glucose, retard glucose diffusion, and inhibit the activity of alpha-Amylase and may be responsible for decreasing the rate of glucose absorption and concentration of postprandial serum glucose; and
- (vi) Terpenoids and Steroids— α -amyrin acetate (fruits of *Ficus racemosa*); andrographolide (leaf of *A. paniculata*); 3 ω -acetoxy-16 c-hydroxybetulinic acid (stem bark of *Zanthoxylum gillettii*); bassic acid (root bark of *Bumelia santorum*); charantin (fruit and seed of *Momordica charantia*); christinin-A (leaf of *Zizyphus spina-christi*); colosolic acid, maslinic acid (leaf of *Lagerstroemia speciosa*); corosolic acid (leaf of *Vitex* spp.); elatosides E (root cortex of *Aralia elata*); escins-IIA and IIB (seeds of *Aesculus hippocastanum*); forskolin (*Coleus forskohlii*); ginsenosides (rhizome of *Panax* sp.); gymnemic acid IV (leaf of *Gymnema sylvestre*); momordin ic (fruit of *Kochia scoparia*); β -sitosterol (*A. indica*); senegin derivatives (*Polygala senega*);
- (vii) Saponin, (Triterpenoid + steroidal)—Stimulates the release of insulin and related compounds;
- (viii) Glycosides—Kalopanax (stem bark of *Kalopanax pictus*); jamboline/ antimellin (seeds of *S. cumini*); myrciacitrins I and II and myrciaphenones A and B (leaf of *Myrcia multiflora*); neomyrtillin (leaf of *Vaccinium myrtillus*); pelargonidin 3-*O*- α -L rhamnoside (bark of *F. bengalensis*); pseudoprotinosaponin AIII and protinosaponin AIII (rhizome of *A. asphodeloides*); vitexin, isovitexin and isorhamnetin 3-*O*- β -D-rutinoside (leaf of *Microcos paniculata*);
- (ix) Miscellaneous compounds—allicin (bulb of *A. sativum* and *Allium cepa*); bellidifolin (*Swertia japonica*); bakuchiol (*Otholobium pubescens*); curcuminoids (rhizome of *C. longa*); ellagitannins (fruit of *T. chebula*);

Some of the antidiabetic plants have insulin mimetic or insulin secretory activity (Table 7.7).

Table 7.7 List of plants having insulin mimetic or insulin secretory activity and their mechanism of action

S. no.	Plant botanical name	Family	Mechanism of action
1	<i>Abies pindrow</i>	Pinaceae	Insulin secretagogue activity
2	<i>Acacia arabica</i>	Leguminosae	Release of insulin from pancreas
3	<i>Agrimony eupatoria</i>	Leaves	Insulin releasing and insulin like activity
4	<i>Aloe barbadensis</i>	Liliaceae	Stimulating synthesis and release of insulin
5	<i>Annona squamosa</i>	Annonaceae	Increased plasma insulin level
6	<i>Averrhoa bilimbi</i>	Oxalidaceae	Increase serum insulin level
7	<i>Bixa orellana</i>	Bixaceae	Increase plasma insulin concentration and increase insulin binding on insulin receptor
8	<i>Boerhavia diffusa</i>	Nyctaginaceae	Increase plasma insulin concentration
9	<i>Camellia sinensis</i>	Theaceae	Increase insulin secretion
10	<i>Capsicum frutescens</i>	Solanaceae	Increase insulin secretion and reduction of insulin binding on the insulin receptor
11	<i>Cinnamomum zeylanicum</i>	Lauraceae	Elevation in plasma insulin level
12	<i>Clausena anisata</i>	Rutaceae	Stimulate secretion of insulin
13	<i>Eucalyptus globulus</i>	Myrtaceae	Increase insulin secretion from clonal pancreatic beta line (BRIN-BD 11)
14	<i>Ficus religiosa</i>	Moraceae	Initiating release of insulin
15	<i>Hibiscus rosasinensis</i>	Malvaceae	Stimulate insulin secretion from beta cells
16	<i>Helicteres isora</i>	Sterculiaceae	Decrease plasma triglyceride level and insulin sensitizing activity
17	<i>Ipomoea batata</i>	Convolvulaceae	Reduce insulin resistance and blood glucose level
18	<i>Juniperus communis</i>	Pinaceae	Increase peripheral glucose consumption and induce insulin secretion
19	<i>Olea europaea</i>	Oleaceae	Increase insulin release and increase peripheral uptake of glucose
20	<i>Swertia chirata</i>	Gentianaceae	Stimulates insulin release from islets

(continued)

Table 7.7 (continued)

S. no.	Plant botanical name	Family	Mechanism of action
21	<i>Scoparia dulcis</i>	Scrophulariaceae	Insulin-secretagogue activity
22	<i>Tinospora crispa</i>	Menispermaceae	Anti-hyperglycemic, stimulates insulin release from islets
23	<i>Urtica dioica</i>	Urticaceae	Increase insulin secretion
24	<i>Vinca rosea</i>	Apocynaceae	Beta cell rejuvenation, regeneration and stimulation
25	<i>Zingiber officinale</i>	Zingiberaceae	Increase insulin level and decrease fasting glucose level

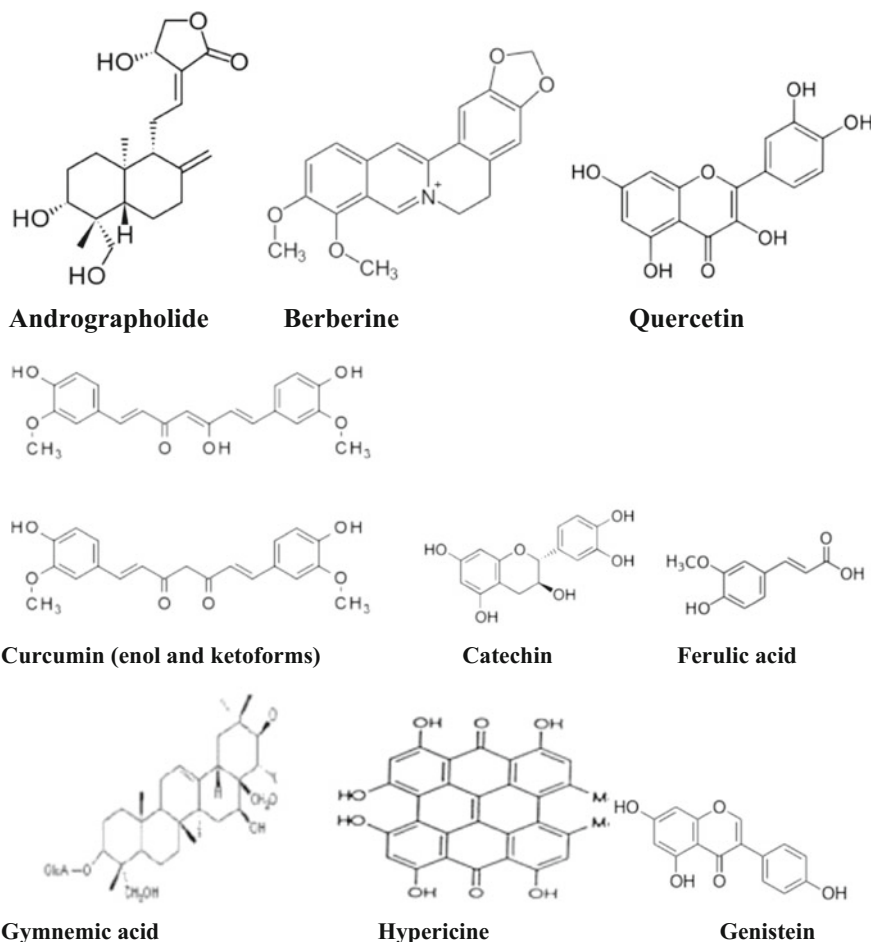
Bioactive compounds having insulin secretagogues or insulin mimetic activity, however, may be different in different plants (Table 7.8).

Structures of some phytoconstituents with hypoglycemic activity are given in Fig. 7.2.

Medicinal plants or the bioactive compounds in their extracts show glucose-lowering either through insulin-mimicking activity, enhanced β -cells regeneration, or glucose uptake. Phytoconstituents like alkaloids (as major role of alkaloids) inhibits alpha-glucosidase and decrease glucose transport through the intestinal epithelium; imidazoline compounds stimulate insulin secretion in a glucose-dependent manner; polysaccharides increase the level of serum insulin, reduced the blood glucose level of serum insulin, reduce the blood glucose level, and enhance tolerance to glucose; flavonoids suppress the glucose level, saponin stimulates the release of insulin, and blocks the formation of glucose in the bloodstream (Bhushan et al. 2010). The antidiabetic principles of bitter melon are a mixture of steroidal saponins, insulin-like peptides, alkaloids, and triterpenoids. Alkaloids such as aconitine, anisodamine, charantine, and leurosine, showed antidiabetic effects. Dietary flavonoids and acarbose synergistically inhibit α -glucosidase and lower postprandial blood glucose.

Hypertension (HTN) management

Hypertension (HTN) or high blood pressure (BP) is a chronic medical condition in which the BP in the arteries is elevated. BP is a combination of systolic and diastolic pressure. According to WHO criteria, hypertension means elevated blood pressure levels above 140/90 mmHg (Lifton 1996). Systolic pressure represents blood force, or pressure, while the heart is beating and diastolic pressure stands for blood pressure when the heart is at rest. Systolic pressure is always the first or top measurement in a blood pressure reading. In a reading of 130/80, 130 represents systolic pressure and 80 represents diastolic pressure. In prehypertension, systolic numbers range from 120 to 129 and diastolic numbers are less than 80. HTN has been named the “silent killer” as it is asymptomatic and the major contributor or risk factor for cardiovascular morbidity and mortality (Gavras 2009). In 2000,



	R ₁	R ₂
Gymnemic acid 1:	Tigloyl	Ac
Gymnemic acid 2:	2-Methylbutyloyl	Ac
Gymnemic acid 3:	2-Methylbutyloyl	H
Gymnemic acid 4:	Tigloyl	H

Fig. 7.2 Showing structure of phytoconstituents having insulin insulin mimetic activity

26.4% of the world's population suffered hypertension and it is predicted that this rate would increase by 60% in 2025 (Kearney et al. 2005).

Ranges of BP are (i) normal: less than 120/80 mmHg, (ii) prehypertension: systolic between 120–129 and diastolic <80, (iii) stage 1 high blood pressure:

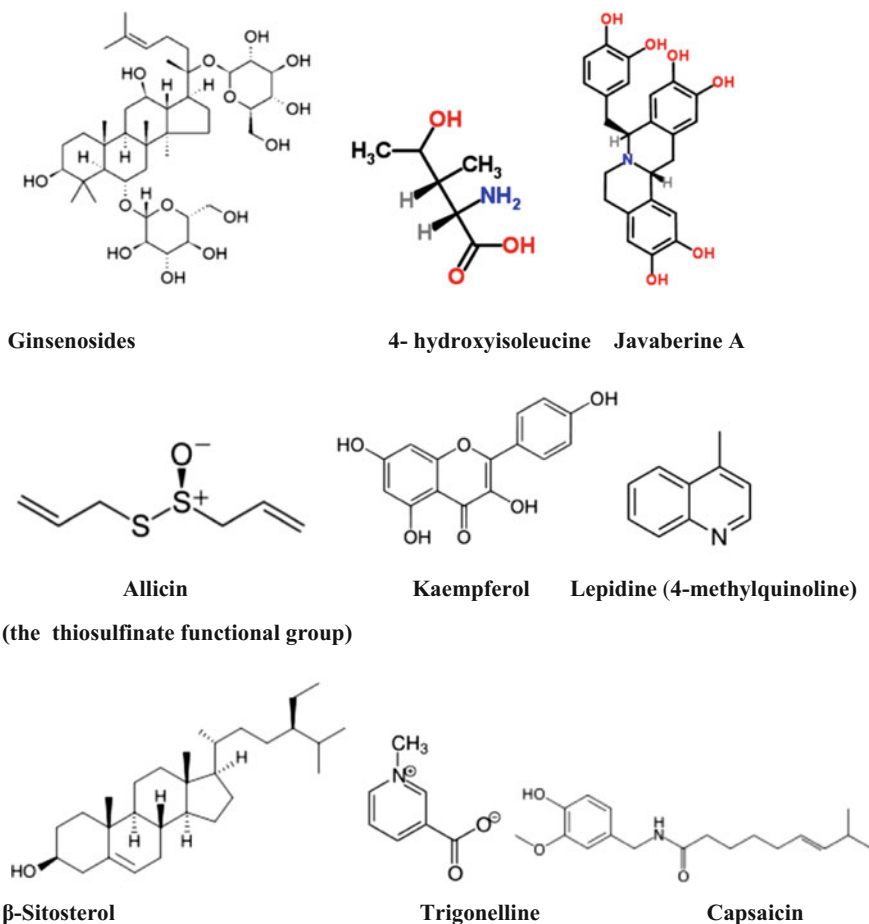


Fig. 7.2 (continued)

systolic between 130 and 139 or diastolic between 80 and 89, (iv) stage 2 high blood pressure: systolic at least 140 or diastolic at least 90 mmHg. According to Barker et al. (1995), the categories of hypertension in adults may be different (Table 7.9).

Although both numbers (systolic and diastolic readings) are significant, after age ~50, the systolic number is most important and only 10% of high blood pressure cases are due to secondary or identifiable causes such as medications, or conditions and diseases of other organs. High blood pressure happens when the pressure on the arteries and blood vessels becomes too high and the arterial wall becomes distorted causing extra stress on the heart. Long-term high blood pressure increases the risk of stroke, heart attack, and diabetes. Results of high blood pressure include arterial damage, aneurysm, heart failure, blocked or ruptured blood

Table 7.8 List of plants and their constituents having insulin secretagogues or insulin mimetic activity

S. no.	Plant botanical name	Family	Active constituents
1	<i>Aloe vera</i>	Liliaceae	Pseudoprotinosaponin AIII and protinosaponins AIII
2	<i>Anemarrhena asphodeloides</i>	Liliaceae	Mangiferin and mangiferin-7-O-β-D' glucoside
3	<i>Bauhinia variegata</i>	Caesalpiniaceae	Roseoside
4	<i>Camellia sinensis</i>	Theaceae	Epigallocatechin gallate
5	<i>Citrullus colocynthis</i>	Cucurbitaceae	Beta-pyrazol-1-ylalanine
6	<i>Ephedra distachya</i>	Ephedraceae	L-ephedrine
7	<i>Eriobotrya japonica</i>	Rosaceae	Cinchonain ib
8	<i>Eugenia jambolana</i>	Myrtaceae	Pandanus odoros (Toei-hom) a 4-hydroxybenzoic acid
9	<i>Ficus benghalensis</i>	Moraceae	Leucocyanidin 3-O-beta-D-galactosyl cellobioside, leucopelargonidin-3-O-alpha-L rhamnoside
10	<i>Glycyrrhizae radix</i>	Fabaceae	Glycyrrhetic acid, dihydroxy gymnemic triacetate
11	<i>Momordica charantia</i>	Cucurbitaceae	Momordicin, charantin, and galactose-binding lectin
12	<i>Panax ginseng</i>	Araliaceae	Polypeptides
13	<i>Prunella vulgaris</i>	Labiatae	Jiangtang su
14	<i>Psidium guajava</i>	Myrtaceae	Strictinin, isostrictinin and pedunculagin
15	<i>Pterocarpus marsupium</i>	Fabaceae	Epicatechin
16	Semen coicis	Gramineae	Coixans
17	<i>Stevia rebaudiana</i>	Asteraceae	Stevioside, steviol
18	<i>Swertia chirayita</i>	Gentianaceae	Swerchirin
19	<i>Teucrium polium</i>	Lamiaceae	Apigenin
20	<i>Trigonella foenum-graecum</i>	Leguminosae	4-hydroxyleucine and hydroxyisoleucine
21	<i>Zizyphus spina-christi</i>	Rhamnaceae	Christinin-A

Table 7.9 Categories of hypertension in adults (measured in mmHg)^a

Stages	Systolic BP range	Diastolic BP range
Stage 1 (mild)	Systolic BP 140–159	Diastolic BP 90–99
Stage 2 (moderate)	Systolic BP 160–179	Diastolic BP 100–109
Stage 3 (severe)	Systolic BP 180–209	Diastolic BP 110–119
Stage 4 (very severe)	Systolic BP >210	Diastolic BP >120

^aBarker et al. (1995)

vessels, reduced kidney function, vision loss, loss of cognitive function (concentration, memory, and ability to learn), metabolic syndrome (high cholesterol and insulin, atherosclerosis and increased waist size), etc. Frequently, there are no symptoms as blood pressure increases, but warning signs for very high BP include usually chest pains, confusion, headaches, ear noise or buzzing, irregular heartbeat, nosebleeds, tiredness or vision changes, etc. High-salt diet, emotional stress, alcohol, caffeine, smoking, obesity, inactivity, birth control pills, heavy-metal poisoning, etc., are the probable causes of high blood pressure. Patients with BP higher than 130/80 mmHg with concomitant presence of diabetes or kidney disease require further treatment. Exercise HTN is an excessively high elevation (systolic values between 200 and 230 mmHg) in BP during exercise. Exercise HTN may indicate that an individual is at risk for developing HTN at rest. Persistent HTN is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure. Moderate elevation of arterial BP leads to shortened life expectancy (Table 7.10). Different causes of hypertension are described in Table 7.8.

Recent evidence indicate that hypertension and raised blood pressure are increasing partly because of the increase in risk factors including smoking, obesity, and high use of alcohol, social insecurity and anxiety, and lack of exercise. Both dietary and lifestyle changes as well as medicines can improve BP control and decrease the risk of associated health complications.

Conventional antihypertensives are usually associated with many side effects. Conventional medicines used in the treatment of hypertension are classified as follows:

- (i) Diuretics—diuretics help the kidneys eliminate excess salt and water from the body's tissues and blood and include loop diuretics (bumetanide, ethacrynic acid, furosemide, torsemide); thiazide diuretics (epitizide, hydrochlorothiazide and chlorothiazide, bendroflumethiazide, methyclothiazide, polythiazide); thiazide-like diuretics (indapamide, chlorthalidone, metolazone); potassium-sparing diuretics (amiloride, triamterene, spironolactone, eplerenone);
- (ii) Adrenergic receptor antagonists—beta blockers (atenolol, bisoprolol, betaxolol, carteolol, carvedilol, labetalol, metoprolol, nadolol, nebivolol, oxprenolol, penbutolol, pindolol, propranolol, timolol, etc.); alpha blockers (doxazosin, phentolamine, indoramin, phenoxybenzamine, prazosin,

Table 7.10 Various causes of hypertension

Hypertension type	Cause
Primary hypertension (essential hypertension)	Increased sympathetic nervous system activity Increased production of sodium-retaining hormones and vasoconstrictors Deficiencies of vasodilators such as prostacyclin and nitric oxide Inappropriate or increased renin secretion, resulting in increased production of angiotensin II and aldosterone. Genetic predisposition
Secondary hypertension	Renal: acute glomerulonephritis, chronic nephritis, polycystic disease, diabetic nephropathy and hydronephrosis Endocrine: Acromegaly, Hypothyroidism, Hyperthyroidism, Hypercalcaemia (hyperparathyroidism) Adrenal: Cortical: Cushing syndrome, primary aldosteronism, congenital adrenal hyperplasia, apparent mineralocorticoid excess (licorice) Medullary: Pheochromocytoma, extra-adrenal chromaffin tumors, Carcinoid Exogenous hormones: estrogen, glucocorticoids, mineralocorticoids, sympathomimetics, tyramine-containing food, monoamine oxidase inhibitors Systolic hypertension: Increased cardiac output Aortic valvular insufficiency, Arteriovenous fistula, patent ductus arteriosus Thyrotoxicosis, Rigidity of aorta Iatrogenic hypertension Pregnancy-induced hypertension Neurological disorders: Increased intracranial pressure –brain tumors –encephalitis –respiratory acidosis

terazosin, tolazoline); and mixed alpha and beta blockers (bucindolol, carvedilol, labetalol);

- (iii) Adrenergic receptor agonists—centrally acting adrenergic drugs or alpha-2 adrenergic receptor agonist—clonidine, guanabenz, guanfacine, methyl-dopa, moxonidine;
- (iv) Calcium channel blockers—calcium channel blockers block the entry of calcium into muscle cells in artery walls and include dihydropyridines (amlodipine, lacidipine, cilnidipine, clevidipine, felodipine, isradipine, lercanidipine, levamlodipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine) and non-dihydropyridines (diltiazem, verapamil);
- (v) ACE inhibitors—ACE inhibitors inhibit the activity of angiotensin-converting enzyme (ACE), an enzyme responsible for the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor. Examples include captopril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, trandolapril, benazepril, etc.;

- (vi) Angiotensin II receptor antagonists—Angiotensin II receptor antagonists work by antagonizing the activation of angiotensin receptors (e.g., azilsartan, candesartan, eprosartan, irbesartan, losartan, olmesartan, olmesartan medoxomil, telmisartan, valsartan, fimasartan, etc.);
- (vii) Aldosterone antagonists—eplerenone, spironolactone; and
- (viii) Vasodilators—sodium nitroprusside, hydralazine.

Figure 7.3 showing structure of some conventional antihypertensives drugs that are used to treat hypertension.

Many of these antihypertensive agents have some side effects, e.g., diuretics may cause muscle cramps, dizziness, extreme tiredness, dehydration, blurred vision, abnormal heart rate, skin rash, and others; ACE inhibitors may cause cough, skin rash, vomiting, kidney failure, fever, sore throat, diarrhea, and others; and side effects come with the use of calcium channel blockers are fatigue, headache, diarrhea, constipation, skin rash, edema, etc. Herbs do not cause side effects like weakness, tiredness, drowsiness, impotence, cold hands and feet, depression, insomnia, abnormal heartbeats, skin rash, dry mouth, dry cough, stuffy nose, headache, dizziness, swelling around eyes, constipation or diarrhea, fever, etc. Herbal medicines have been gaining more attention in the treatment of hypertension both in the developed and developing countries because of their wide biological and medicinal activities, diversity, ease of availability, higher safety margins and low cost than novel pharmaceuticals. In the past few decades, a lot of concerted efforts have been channeled into researching the local plants with antihypertensive therapeutic values and a lot of concerted efforts have been channeled into researching into local plants with antihypertensive therapeutic values, in which some of these medicinal plants have been validated and others disproved. Many of the following medicinal plants have so far been scientifically studied and reported to have hypotensive or antihypertensive effects:

Ajwain (*Carum copticum*), American Ginseng (*Panax quinquefolius*), Amur Cork Tree (*Phellodendron amurense*), Arjuna (*T. arjuna*), Asafetida (*Ferula assafoetida*), Ashwagandha (*W. somnifera*), Avocado (*Persea americana*), Banana (*Musa sapientum*), Barberry (*B. vulgaris*), Basil (*Ocimum basilicum*), Black bean (*Castanospermum australe*), Black cumin (*N. sativa*), Black Mangrove (*Lumnitzera racemosa*), Black plum (*Vitex doniana*), Black Walnut (*Juglans nigra*), Bilberry (*V. myrtillus*), Biting Stonecrop (*Sedum acre*) Borage (*Borago officinalis*), Breadfruit (*Artocarpus altilis*), Broccoli (*Brassica oleracea*) Buchu (*Agathosma betulina*), Cantaloupe or musk melon (*Cucumis melo*), Cardamon (*Elettaria cardamomum*), Carrot (*Daucus carota*), Cat's Claw herb (*Uncaria tomentosa*), Celery seed (*Apium graveolens* var. *dulce*), Chaksu (*Cassia absus*), Chicory (*Cichorium intybus*), Chinese Knotweed or Fo-Ti (*Polygonum multiflorum*), Chocolate or cocoa bean (*Theobroma cacao*), Cicely (*Myrrhis odorata*), Cinnamon (*Cinnamomum verum* or *C. tamala*), Coconut root (*Cocos nucifera*), Coffee weed (*Cassia occidentalis*), Custard apple (*Annona reticulata*), Dandelion (*Taraxacum officinale*), Dodder (*Cuscuta reflexa*), Dong Quai (*Angelica sinensis*), Flaxseed (*Linum usitatissimum*), French Lavender (*Lavandula stoechas*), Hyssop (*Hyssopus officinalis*), Juniper—

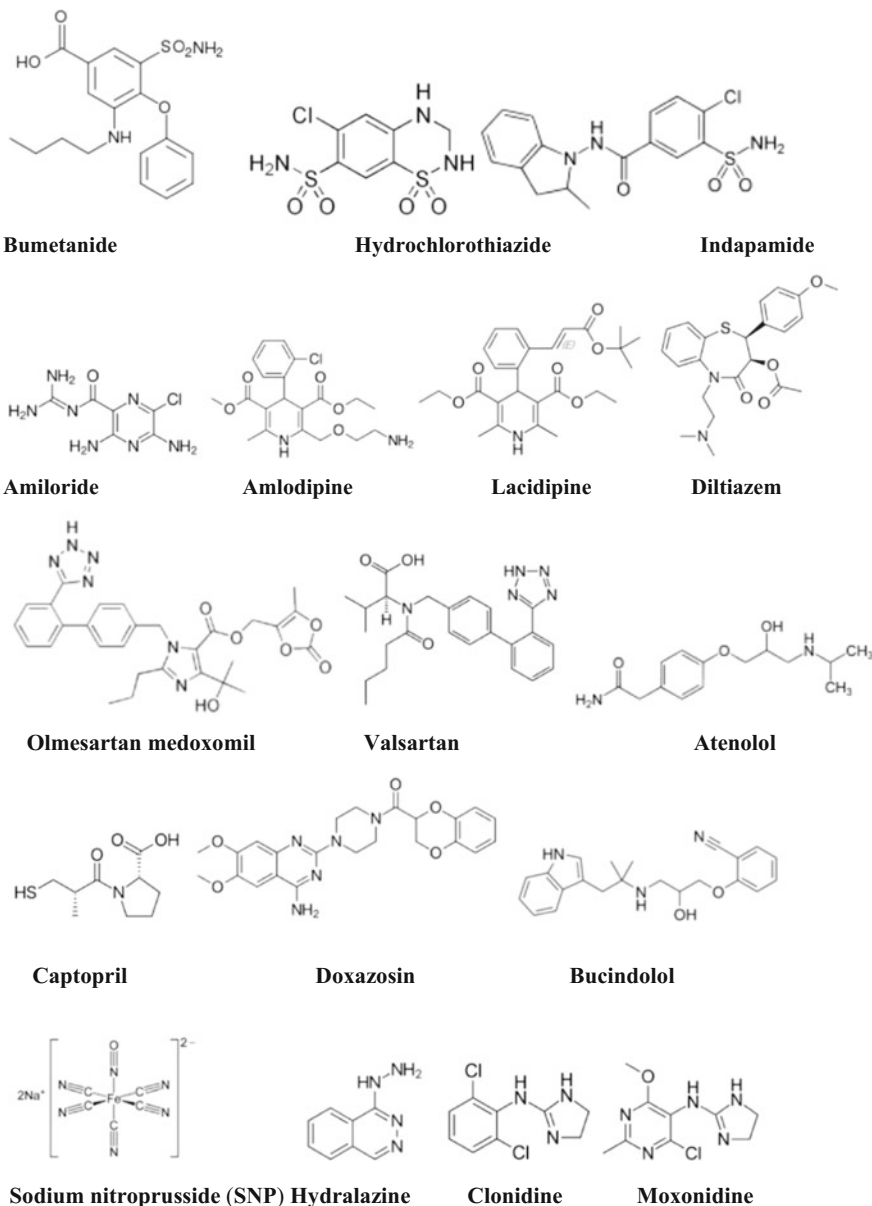


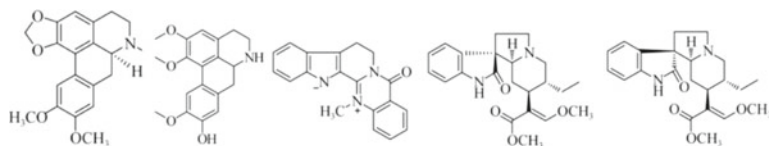
Fig. 7.3 Structure of some conventional antihypertensives drugs that are used to treat hypertension

(*Juniperus communis*), Kudzu (*Pueraria lobata*), Lemon Grass—(*Cymbopogon citratus*), Garlic (*A. sativum*), Guan Mu Tong (*Aristolochia manshuriensis*), Ginger (*Zingiber officinale*), Ginseng (*Panax* sp.), Hardy Fuchsia (*Fuchsia magellanica*),

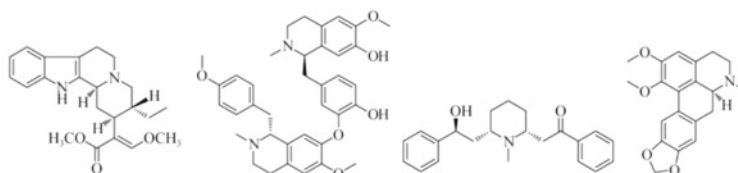
Fig. 7.4 Showing structure of some hypotensive bioactive natural products of secondary metabolic origin. Alkaloids: (+)-Dicentrine, Laurotetanine, Dehydroevodiamine, Rhynchophylline, Isorhynchophylline, Dihydrocorynantheine, Isoliensinine, (-)-Lobeline, (+)-Nantenine, Puqienine A, Puqienine B, Puqienine E, Reserpine, Deserpidine, Tetrandrine; Diterpenes: Forskolin, Stevioside, a diterpenoid glycoside, 14-Deoxy-11, 12-didehydroandrographolide (DDA), a diterpenoid, *ent*-Kaur-16-en-19-oic acid and *ent*-kaur-16-en-15-one-19-oic acid are two kaurane-type diterpenes; Essential oil constituents: α -Pinene, α -Terpinene, γ -Terpinene, p-Cymene, Carvacrol, Thymol, and Linalool; Coumarins: (+)-Praeruptorin A, a coumarin, Imperatorin, a dietary furanocoumarin, Ostruthol, a furanocoumarin; Flavonoids: Quercetin, Isorhamnetin, Astragaln, Orientin, Cardamonin, Alpinetin; flavonoids vicenin-2 (6,8-di- β -D-glucopyranosyl-5,7,4'-trihydroxyflavone), butin (7,3',4'-trihydroxyflavanone), and 3'-hydroxydaidzein (7,3',4'-trihydroxyisoflavone); Others: 33-Daleformis

Harmal (*Peganum harmala*), Hawthorn (*Crataegus* spp.), Chinese Hawthorn (*Crataegus pinnatifida*), Indian plantago (*Plantago* sp.), Karpurvali (*C. forskohlii*), Kudzu (*P. lobata*), Lasaf (*Capparis cartilaginea*), Maritime pine (*Pinus pinaster*), Mistletoe (*Viscum album*), Motherwort (*Leonurus cardiaca*), Murungai leaf (*Moringa oleifera*), Nela nelli (*Phyllanthus amarus*), Oat (*Avena sativa*), Oliver (*Rhaptopetalum coriaceum*), Osbeck (*Desmodium styracifolium*), Parsley (*Petroselinum crispum*), Pau d'Arco (*Tabebuia avellanadae*), Periwinkle (*Vinca minor*), Pima cotton leaf (*Gossypium barbadense*), Pomegranate (*Punica granatum*), Punarnava (*Boerhavia diffusa*), Purslane (*Portulaca oleracea*), Radish (*Raphanus sativus*), Rauwolfia (*Rauwolfia serpentina*), Red Sage (*Salvia miltiorrhiza*), Roselle (*Hibiscus sabdariffa*), Self heal (*Prunella vulgaris*), Sesame (*Sesamum indicum*), Spinach (*Spinacia oleracea*), Soybean (*Glycine max*), Sticky nightshade (*Solanum sisymbriifolium*), Stinging nettle (*Urtica dioica*), Stone breaker (*Lepidium latifolium*), Sunflower (*Helianthus annuus*), Swamp lily (*Crinum gallacum*), Sweet orange (*Citrus sinensis*), Tea (*Camelia sinensis*), Tomato (*Lycopersicon esculentum*), Umbrella tree or cork wood (*Musanga cecropioides*), Virginia dayflower (*Commelina virginica*), Watermelon (*Citrullus lanatus*), Wheat bran (*Triticum aestivum*), Wild tomato (*Solanum sisymbriifolium*), Wild Yam (*Dioscorea villosa*), Yarrow (*Achillea millefolium*). Many of these plants contain bioactive compounds of secondary metabolic origin, fibers, and high-level potassium, or all components. Ren-Ren et al. (2015) while examining the antihypertensive activity of different plant sources such as *Stevia rebaudiana*, *Hippophae ramnoides*, *Fritillaria pugiensis*, *Evodia rutaecarpa*, *C. forskohlii*, *Peucedanum ostruthium*, *Salvia miltiorrhizae*, *Nandina domestica*, *Uncaria rhynchophylla*, and *Musa sapientum*, put emphasis on the research and development of natural lead compounds with antihypertensive activity, including alkaloids, diterpenes, coumarins, flavonoids, and peptides.

Phytochemical screening confirms the presence of (i) alkaloids-(+)-dicentrine, laurotetanine, dehydroevodiamine, rhynchophylline, isorhynchophylline, dihydrocorynantheine, isoliensinine, (-)-lobeline, (+)-nantenine, puqienine A, puqienine B and puqienine E, reserpine, deserpidine, tetrandrine; (ii) saponins; (iii) polyphenols-



(+)-Dicentrine Laurotetanine Dehydroevodiamine Rhynchophylline Isorhynchophylline

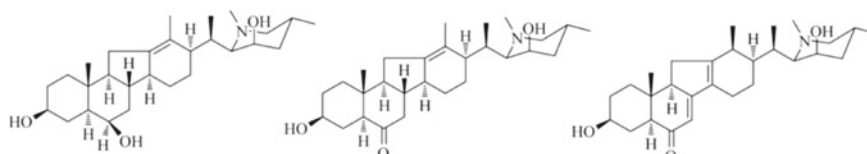


Dihydrocorynantheine

Isoliensinine

(-)-Lobeline

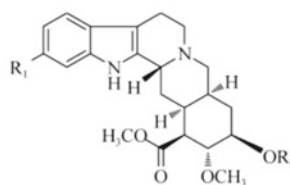
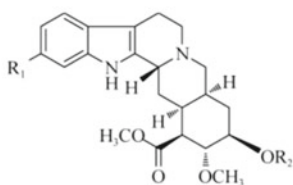
(+)-Nanteni



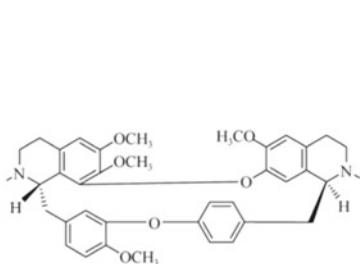
Puqienine A,

Puqienine B

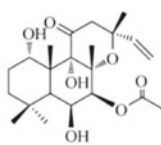
Puqienine E



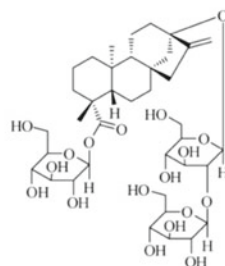
Reserpine (R₁=OCH₃; R₂=3,4,5-trimethoxybenzoyl) Deserpidine (R₁=H; R₂=3,4,5-trimethoxybenzoyl)



Tetrandrine



Forskolol



Stevioside

isochroman-4-one XJP; (iv) phenolic glycosides; (v) flavonoids—quercetin, isorhamnetin, stragalin, orientin, cardamonin, alpinetin; vicenin-2, butin, and 3'-hydroxydaidzein; (vi) coumarins (+)-praeruptorin A, imperatorin, ostruthol; (vii) diterpenes and terpenoids—forskolin, stevioside, 14-deoxy-11,

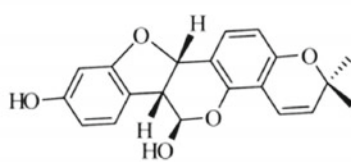
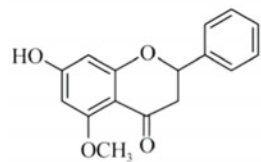
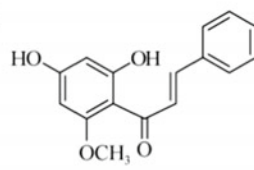
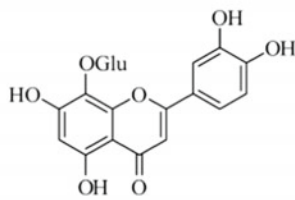
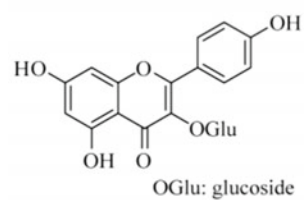
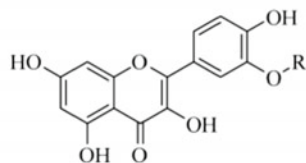
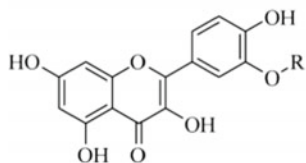
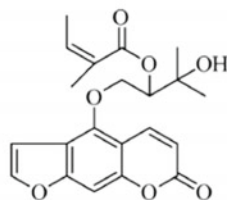
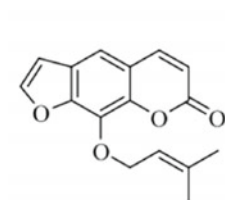
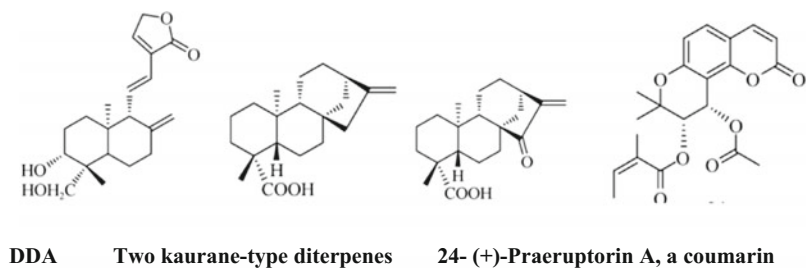


Fig. 7.4 (continued)

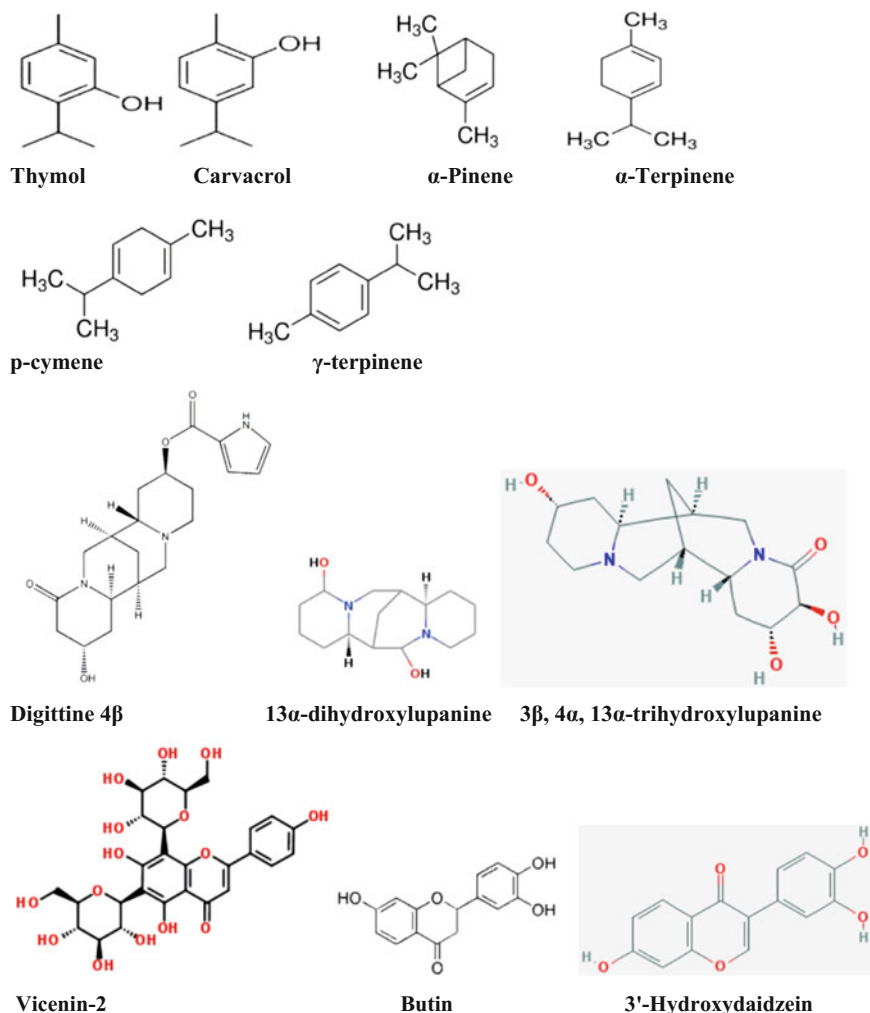


Fig. 7.4 (continued)

12-didehydroandrographolide–DDA; major constituents of essential oil— α -pinene, α -terpinene, γ -terpinene, p-cymene, carvacrol, thymol, and linalool; (viii) anthraquinones; (ix) tannins; (x) phytosterols; (xi) cardiac glycosides; (xii) peptides (Heshiko), and (xiii) others—daleformis, ursolic, moronic acids, Magnesium lithospermate B; are important groups of bioactive compounds, which played a dominant role in the treatment of hypertension. Structure of some of these bioactive antihypertensive compounds are shown below (Fig. 7.4).

Cancer management

Cancer is a class of diseases characterized by out-of-control cell growth. *Cancer* is the uncontrolled growth of abnormal cells in the body. *Cancer* develops when the body's normal control mechanism stops working. Old cells do not die and instead grow out of control, forming new, abnormal cells. These extra cells may form a mass of tissue, called a tumor. Some cancers, such as leukemia, do not form tumors. There are >200 different types of cancer and each is classified by the type of cell that is initially affected. Cancers respond to treatment in different ways, some types of cancer are best treated with surgery, others respond better to drugs (chemotherapy), radiation, etc., and often 2 or more treatments are used to get the best results.

Cancer is divided into five stages using the international TNM system—0, I, II, III, IV (T stands for primary tumor, N for lymph nodes, and M for distant metastases). Stage 0 means the presence of a small carcinoma in situ that has not spread. Stage IV means cancer that has spread widely or cancer that has metastasized. In some cases, the stages have subclasses. When it spreads from the primary site via the bloodstream or lymphatic system to other organs, cancer starts to produce metastases and become attached to other organs. From there they begin to divide and invade space. Different cancers typically metastasize in certain organs, e.g., in the liver, lungs, adrenal glands, brain, and bones. The symptoms caused by metastases vary according to their location.

Medicinal plants have been used to prevent and to treat various diseases and they comprise a source of bioactive pharmaceuticals with beneficial health effects. Certain bioactive components from the plants have been confirmed for their anticancer activities. These include curcumin from turmeric, genistein from soybean, EGCG from green tea polyphenols from green tea, resveratrol from grapes, sulforaphane from broccoli, isothiocyanates from cruciferous vegetables, silymarin from milk thistle, diallyl sulfide from garlic, lycopene from tomato, rosmarinic acid from rosemary, apigenin from parsley, gingerol from gingers, vitamin E from plant oil, boron-rich natural compound, hydroxytyrosol from virgin olive oil, phytoestrogens, etc. Up to now, cancer remains to be one of the leading causes of death in the world and modern drug-targeted therapies has undeniably improved cancer patients' cares, but the advanced metastasized cancer remains untreatable. Cancer chemoprevention with natural bioactive phytochemicals is an emerging strategy to prevent, impede, delay, or cure cancer.

Phytochemicals used as cancer chemopreventive

- (i) Apigenin is a flavone present in vegetables such as parsley, celery, chamomile, and *Moringa peregrina*. It demonstrates cytotoxic activities against breast cancer cell lines (MCF 7), colon cell line (HCT 116), and its cytotoxic activity is comparable to that of doxorubicin;
- (ii) Curcumin (diferuloylmethane) is the major components of popular spice turmeric, *C. longa* L., a member of the ginger family. Its anticancer effects have been studied for colon cancer, breast cancer, lung metastases, and brain tumor;

- (iii) Crocetin from, *Crocus sativus* L. Saffron is a food colorant present in the dry stigmas of the plant and it is a potential agent for a novel anticancer drug against hepatocellular carcinoma;
- (iv) Cyanidin is an extract of pigment from red berries such as grapes, blackberry, cranberry, raspberry, or apples and plums, red cabbage, and red onion. It possesses antioxidant and radical-scavenging effects which may reduce the risk of cancer;
- (v) Indole-3-carbinol (I3C) is found in brassica vegetables, such as broccoli, cauliflower, collard greens. Diindolylmethane (DIM) is a digestion derivative of indole-3-carbinol via condensation formed in the acidic environment of the stomach. Both are studied for their anticarcinogenic effects;
- (vi) Epigallocatechin gallate (EGCG) is the most abundant catechin compounds in green tea. EGCG can be beneficial in treating brain, prostate, cervical, and bladder cancers.
- (vii) Fisetin is a flavone found in various plants such as *Acacia greggii*, *Acacia berlandieri*, *Eurasian smoketree*, parrot tree, strawberries, apple, persimmon, grape, onion, and cucumber Fisetin alleviates aging effects in the yeast or fruit fly, exerts anti-inflammatory effect in LPS-induced acute pulmonary inflammation and anticarcinogenic effects in HCT-116 human colon cancer cells;
- (viii) Genistein is an isoflavone originates from a number of plants such as lupine, fava beans, soybeans, kudzu, and psoralea, and coffee. Functioning as antioxidant and anthelmintic, genistein has been found to have antiangiogenic effects (blocking formation of new blood vessels), and may block the uncontrolled cell growth associated with cancer, most likely by inhibiting the enzymes that regulate cell division and cell survival (growth factors).
- (ix) Gingerol is the active component of fresh ginger with distinctive spicyness. Gingerol has been studied for its anticancerous effects for the tumors in colon, breast and ovarian and pancreas;
- (x) Kaempferol is a natural flavonol isolated from tea, broccoli, witch-hazel, grapefruit, brussels sprouts, apples, etc., and has been studied for pancreatic cancer and lung cancer;
- (xi) Lycopene is a bright red pigment and phytochemical from tomatoes, red carrots, watermelons, and red papayas. It demonstrates antioxidant activity and chemopreventive effects in many studies, especially for prostate cancer;
- (xii) Phenyl isothiocyanate (PEITC), along with sulforaphane from cruciferous vegetables, such as watercress, broccoli, cabbage, etc., have been studied for induction of apoptosis in cell lines. PEITC has shown very strong potency against melanoma. It has been intensively studied for chemoprevention against breast cancer cells, non-small-cell lung cancer, cervical cancer, osteogenic sarcoma U-2 OS, prostate cancer, and myeloma cell lines;

- (xiii) Resveratrol is a natural phenol and can be found in the red grapes skin, peanuts, and in other fruits. It is cancer chemopreventive;
- (xiv) Rosmarinic acid (RA) is a natural antioxidant found in culinary spice and medicinal herbs such as lemon balm, peppermint, sage, thyme, oregano, and rosemary to treat numerous ailments. Rosemary extracts play important roles in anti-inflammation, antitumor, and antiproliferation in various in vitro and in vivo studies;
- (xv) Sulforaphane is an organosulfur compound obtained from cruciferous vegetables such as broccoli, Brussels sprouts, and cabbages. The enzyme myrosinase in GI tract transforms glucoraphanin into sulforaphane upon damage to the plant such as from chewing.
- (xvi) Triterpenoids are biosynthesized in plants by cyclization of squalene, a triterpene hydrocarbon and precursor of all steroids. This group of phytochemicals are subclassified into cucurbitanes, dammaranes, ergostanes, friedelanes, lanostanes, limonoids, lupanes, oleananes, tirucallanes, ursanes, and the list is still growing. Various in vitro and in vivo studies have been conducted for chemoprevention and therapy of breast cancer, and pancreatic cancer using triterpenoids.
- (xvii) Light-exposed mushroom could be an excellent source of Vitamin D. Vitamin D has been involved in breast cancer, colon cancer, ovarian cancer, and pancreatic cancer;
- (xviii) Vitamin E includes both tocopherols and tocotrienols, a fat-soluble antioxidant, and exists in many foods including wheat germ oil, sunflower oil, and safflower oils. Alphanatocopherol is the most bioactive form of vitamin E that stops the production of reactive oxygen species (ROS) when fat undergoes oxidation. There are reports that both tocopherols and tocotrienols have antitumor effects due to their antioxidant properties, and tocotrienols show stronger bioactivity and both show antiproliferative, proapoptotic, and COX-2 inhibiting effects in in vitro studies.

Plant-derived compounds have been an important source of several clinically useful anticancer agents (Cragg and Newman 2005). These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (Taxol). A number of promising new agents are in clinical development based on selective activity against cancer-related molecular targets, including flavopiridol and combretastatin A4 phosphate, while some bioactive compounds which failed in earlier clinical studies are stimulating renewed interest. Different bioactive compounds respond differently to different types of cancers (Table 7.11).

The mechanisms of action of plant-derived anticancer drugs are numerous and most of them induce apoptotic cell death. Chemotherapy is the treatment of cancer cells with one or more antineoplastic cytotoxic agents, inhibitor, antimetabolic, and anti-microtubule, which mainly targets the rapidly proliferating cancer cells leading to the induction of cell death. Figure 7.5 showing structure of some bioactive herbal anticancer agents.

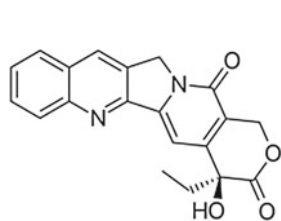
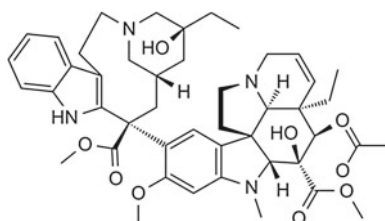
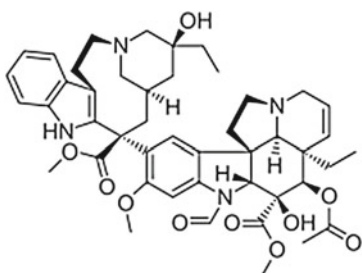
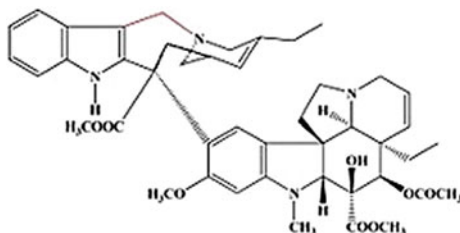
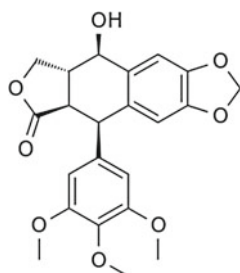
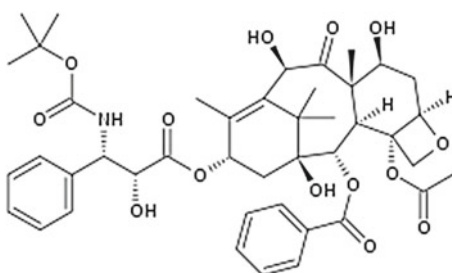
Table 7.11 Influence of different bioactive compounds in different cancers

Sl. no.	Metabolites	Groups	Plant species	Type of cancer
1	Cucumin	Phenolic	<i>Curcuma longa</i>	Colorectal
2	Phenol	Phenolic	<i>Zingiber officinale</i>	Cancer
3	Resveratrol	Phytoalexin	GrapesVitis	Breast
4	Genistein	Flavonoids		Leukemia
5	Biocalcin	Flavonoids	Shosiko	Hepatocellular
6	Hydroxystaurosporin	Alkaloid	<i>Viscom album</i>	Ovarian cancer
7	Lectin	Lectins	Banana	Cancer
8	Xanthorrhizol	Terpenoids	<i>Curcuma longa</i>	Cancer

Camptothecin (CPT) is a topoisomerase inhibitor, isolated from the bark and stem of *Camptotheca acuminata*; *Vinca* alkaloids are a set of antimetabolic and anti-microtubule alkaloid agents originally derived from the periwinkle plant *C. roseus*, include vinblastine, vincristine, vindesine, and vinorelbine. Additional researched *vinca* alkaloids include vinca minor, vincristine, and vinburnine; podophyllotoxin (PPT) is a non-alkaloid toxin lignan extracted from the roots and rhizomes found in *P. peltatum*; *T. brevifolia* (Pacific yew) have been used as the basis for two chemotherapy drugs, docetaxel and paclitaxel; ingenol mebutate (ingenol-3-angelate) is a substance found in the sap of the plant *Euphorbia peplus* and an inducer of cell death. Trastuzumab emtansine (Kadcyla) is an antibody conjugated to a synthetic derivative of the cytotoxic principle of the Ethiopian plant *Maytenus ovatus*. It used to treat breast cancer.

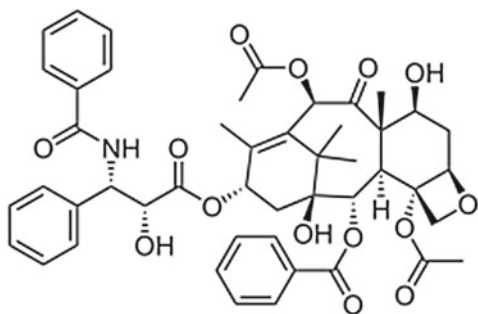
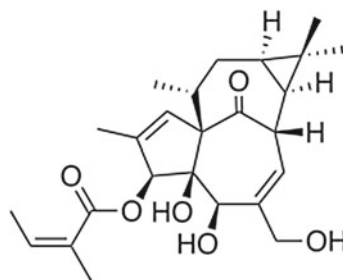
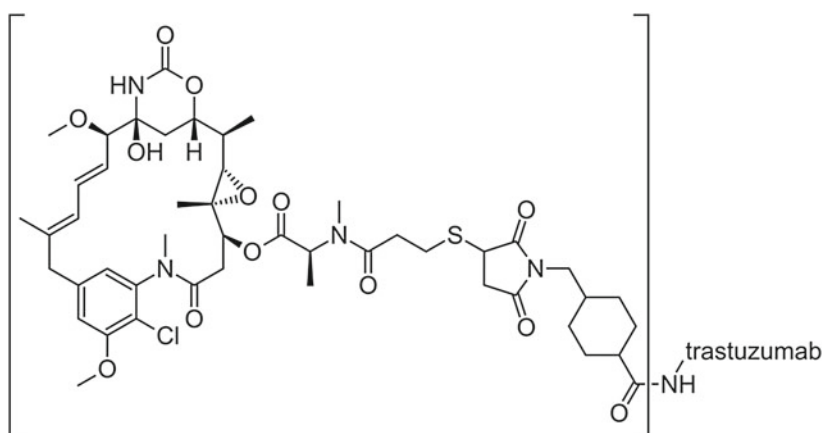
7.3.2 Viral Disease Management with the Use of Antiviral Bioactive Phytoconstituents

Viruses are acellular particulate matter made up of nucleic acids and proteins. They cause many infectious viral diseases including HIV, Influenza, Herpes simplex virus (HSV), dengue, chikungunya, Zika, hepatitis A (HSV), hepatitis B (HSB), hepatitis C (HCV), etc., and recent outbreaks in the advent of globalization and ease of travel have underscored their prevention as a critical issue in safeguarding public health. Despite the progress made in immunization and drug development, many viruses lack preventive vaccines and efficient antiviral therapies, which are often beset by the generation of viral escape mutants. Viral diseases pose a great risk to human health as viral infections are tough to control due to mutative nature of the viral genomes. Medicinal plants provide diverse bioactive phytochemicals which play synergetic role in maintaining human health and there is the constant emergence of new resistant viral strains which demands novel antiviral agents with fewer side effects and cell toxicity (Kapoor et al. 2017). So, identifying novel antiviral drugs is of critical importance and natural products are an excellent source for such

**Camptothecin (CPT)****Vinblastine****Vincristine****Vinorelbine****Podophyllotoxin (PPT)****Docetaxel****Fig. 7.5** Showing structure of some anticancer bioactive herbal agents

discoveries. Polyphenols, alkaloids, flavonoids, saponins, quinones, terpenes, proanthocyanidins, lignins, tannins, polysaccharides, steroids, thiosulfonates, and coumarins are prominent bioactive phytochemicals, which have been observed to combat viral infections.

Lin et al. (2014) summarize the antiviral activities from several natural products and herbal medicines against some notable viral pathogens including coronavirus (CoV), coxsackievirus (CV), dengue virus (DENV), enterovirus 71 (EV71),

**Paclitaxel (PTX)****Ingenol mebutate****Trastuzumab emtansine (Kadcyla)****Fig. 7.5** (continued)

hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus, human immunodeficiency virus (HIV), influenza virus, measles virus (MV), and respiratory syncytial virus (RSV) and found that natural products and herbal ingredients possessed high antiviral activity.

Many antiviral bioactive phytoconstituents such as polysaccharides, lectins, proteins, alkaloids, terpenes, flavonoids, polyphenols, etc., are useful as antiviral agents. Their working mechanisms are different, e.g., polysaccharides inhibit viral replication and viral binding to cell; lectins from banana inhibit virus penetration (HIV), reverse transcriptase and N-glycohydrolases; proteins (GAP31) inhibit viral DNA integration and viral replication, panaxagin reverse transcriptase and inhibit

viral protein synthesis; alkaloids block virus binding, inhibit virus growth, reduce viral titers in lungs (HIV, HSV); terpenes (saponins) inhibit the virus replication; flavonoids are inhibitory on reverse transcriptase, blocking RNA synthesis (HIV, HSV, influenza); polyphenols inhibit the viral cell entry by modulating the viral surface structure and affect the expression of virus proteins on cell surface, etc.

Many medicinal plants possess significant antiviral properties owing to the presence of a large array of different bioactive molecules in them. Researchers are in favor of the use of less toxic antiviral bioactive molecules from natural sources instead of using nucleic acid analogs, protease inhibitors or other toxic synthetic molecules as antiviral therapeutics. Polyphenols, alkaloids, flavonoids, saponins, quinones, terpenes, proanthocyanidins, lignins, tannins, polysaccharides, steroids, thiosulfonates, and coumarins are prominent bioactive phytochemicals, which have been observed to combat viral infections. Examples of some other bioactive antiviral phytochemical agents from plant sources include chalcones (ketone), spiroketal-enol (ether derivative), honokiol, limonoids (lignin), Swerilactones (lactones), xanthohumol (chalcone), decanoylphorbol-13 acetate (diterpene), oleanane, dammarenolic acid and saikosaponins (triterpene and triterpenoid), excoecarianin, loliolide (tannins), jubanines (alkaloids), quercetin (flavonoid), sennoside a (glycoside), silvestrol (benzofuran), sjp-1-5 (ligningomisin), etc. Figure 7.6 showing molecular mechanism of action of antiviral phytochemicals.

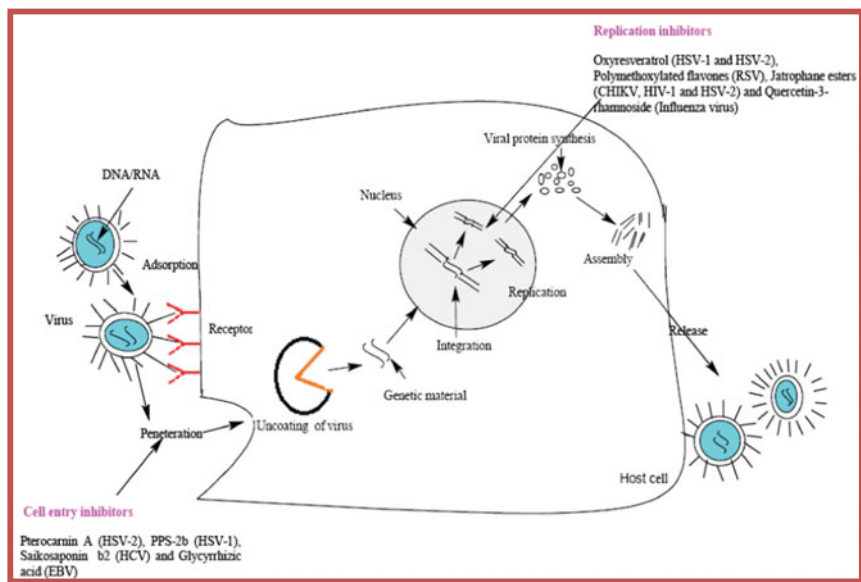
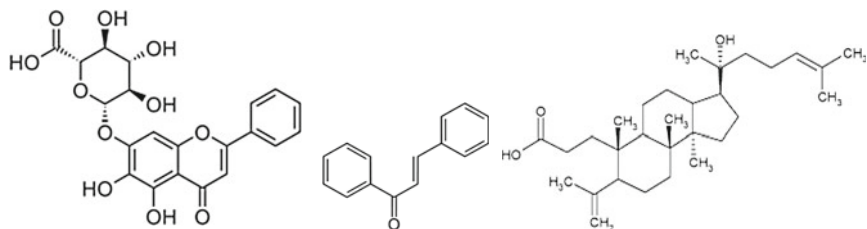
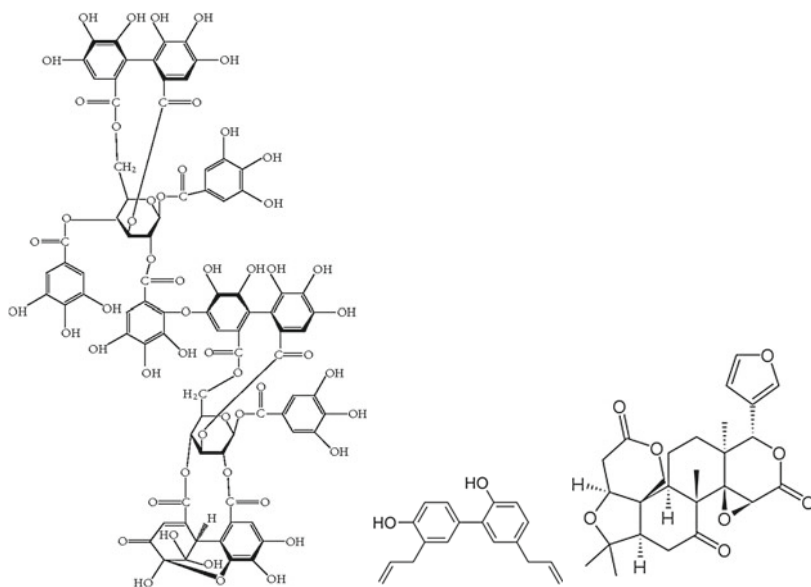
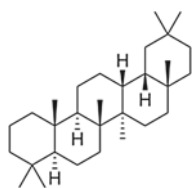
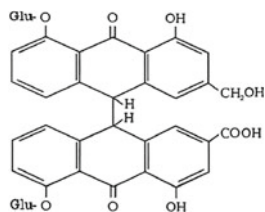


Fig. 7.6 Molecular mechanism of action of antiviral phytochemicals. Adsorption of the virus on to the surface of the target cell→entry of the uncoated virus particle into host cell→replication→assembly→release. Adsorption of the virus on to the surface of the target cell may be inhibited by phytomolecules such as Pterocarinin A, PPS-2b, Saikosaponin glycyrrhizic acid; replication process may be inhibited by some of the phytomolecules such as Oxyresveratrol, Polymethoxylated Flavonoids, Jatrophone esters, and Quercetin or its derivatives

Table 7.12 Some antiviral bioactive compounds from medicinal plants with name of the chemicals, class and their activity

Phytochemicals	Class	Active against virus	Plant/plant parts
Baicalin	Flavone glycoside	DENV	<i>Scutellaria baicalensis</i> (roots)
Chalcones	Aromatic ketone	Influenza A (H1N1)	<i>Glycyrrhiza inflata</i> (roots)
Dammarenolic acid	Triterpenoid	Retroviruses	<i>Aglaia</i> sp. (bark)
Decanoylphorbol-13 acetate	Diterpene	CHIKV	<i>Croton mauritianus</i> (leaves)
Excoecarianin,	Tannins	HSV-2, HCV	<i>Phyllanthus urinaria</i> (whole plant)
Honokiol	Lignan	DENV-2	Magnolia tree (roots, bark)
Jubanines	Cyclopeptide alkaloids	PEDV	<i>Ziziphus jujuba</i> (roots)
Limonoids	Lignin	HCV	<i>Swietenia macrophylla</i> (stem)
Oleanane	Triterpenes	PDEV	<i>Camellia japonica</i> (flowers)
Quercetin	Flavonoid	HCV	<i>Embelia ribes</i> (seeds)
Saikosaponins	Terpenoid	HCV	<i>Bupleurum kaoi</i> (roots)
Sennoside A	Glycoside	HIV-1	<i>Rheum palmatum</i> (roots)
Silvestrol	Benzofuran	Ebola virus	<i>Aglaia foveolata</i> (leaves, bark)
SJP-L-5	Ligningomisin	HIV-1	<i>Schisandra micrantha</i> (roots)
Spiroketal-enol	Ether	HSV-1 HSV-2	<i>Tanacetum vulgare</i> (rhizome)
Swerilactones	Lactones	HBV	<i>Swertia mileensis</i> (whole plant)
Xanthohumol	Chalcone	BVDV	<i>Humulus lupulus</i> (whole plant)

Some antiviral bioactive compounds from medicinal plants with the name of the chemicals, class, their activity, and structure have been given above (Table 7.12; Fig. 7.7). Baicalin is a flavone glycoside. It is the glucuronide of baicalein found in several species in the genus *Scutellaria*, including *Scutellaria baicalensis* and *Scutellaria lateriflora*. Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Honokiol is a lignan isolated from the bark, seed cones, and leaves of trees belonging to the genus *Magnolia*.

**Baicalin****Chalcone****Dammarenolic acid****Excoecarianin****Honokiol****Limonoids****Oleanane (natural triterpene) Sennoside-A (senna glycoside or senna)****Fig. 7.7** Structure of some antiviral bioactive compounds derived from medicinal plants

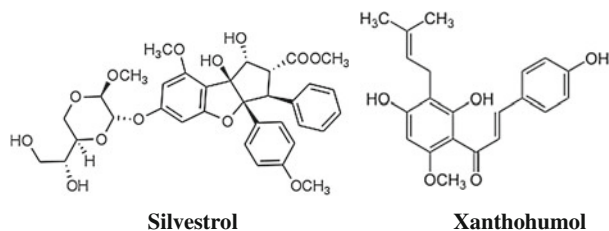


Fig. 7.7 (continued)

7.4 Natural Immunopotentiators, Vaccine and Biotechnology in Health Care

Medicinal plants are now widely used worldwide to address a variety of diseases for their prevention and treatment. Disease prevention is categorized into three levels such as (i) primary prevention (intending to decrease the number of new cases of a disorder or illness by health promotion/education, immunization); (ii) secondary prevention (intending to lower the rate of established cases or prevalence of illness by screening and prompt management); and (iii) tertiary prevention (intending to decrease the amount of disability associated with an existing disorder by rehabilitation and other means).

Disease prevention focuses on strategies that reduce the risk of disease, identify risk factors, or detect disease in its early, most treatable stages and examples of disease prevention activities include regular health examination, immunizations, calcium and Vitamin D supplements to reduce the risk of osteoporosis, blood pressure and assessment of cholesterol and screening for illnesses such as breast, cervical, colorectal and prostate cancer. Medicinal plants play vital roles in disease prevention and their promotion and use fit into all existing prevention strategies and Sofowora et al. (2013) provided a list of >50 plants with potentials in preventive medicine practice. Prevention is better than cure is no doubt the golden rule in health management today and there is a clear need for prevention of a disease developing in the first place. Herbal immunotherapy may be helpful in this regard to reach the desired goal.

7.4.1 *Natural Immunopotentiators and Vaccine Adjuvants from Plants and Other Sources*

Immunopotentiators may be derived from different natural sources such as plants, fungi, marine organisms, and others. Plant-derived immune stimulators consist of a diverse range of small molecules or large polysaccharides, e.g., saponins, tomatine,

inulin, etc., and fungi produce a range of potential candidate molecules, e.g., β -glucans. Other complex molecules that have established adjuvant activity include α -galactosylceramide (from marine sponge), chitosan (from shrimp chitin), and peptides (in bee venom). Some organisms like endophytic fungi and bees, produce immunostimulants using compounds obtained from plants (Woods et al. 2017).

Immunotherapy and herbal immune boosters

Prevention is better than cure and the proverb implies that it is unwise to become diseased and go under treatment to get rid of the curse of the imposed disease rather the disease should be prevented before the attack. It is always safe to prevent the enemy before the attack. Boosting the immune system and prohibiting the entry of microorganisms is better than acting upon them later. Immunotherapy is a medical term defined as the treatment of disease by inducing, enhancing, or suppressing an immune response. Immunotherapy designed to elicit or amplify an immune response are classified as activation immunotherapy, while immunotherapy that reduce or suppress are classified as suppression immunotherapy.

The active agents of immunotherapy are collectively called immunomodulators and they comprise a diverse array of recombinant, synthetic, and natural preparations, often cytokines (Table 7.13). Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod, and cellular membrane fractions from bacteria are already licensed for use in patients. Others including IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG), oligodeoxynucleotides, and glucans are currently being investigated extensively in clinical and preclinical studies. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases.

Cell-based immunotherapies have been proven to be effective for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T-lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of the tumor due to mutation.

Immune booster is a stimulation of the immune system through intake of the vaccine, toxoid (e.g., detoxified bacterial products, which keeps its antigenic properties) or ready to use antibodies (immunoglobulins). The natural active body's immune booster occurs as a result of its infection, and the natural passive immune booster occurs as a result of the maternal antibodies' transport through the placenta

Table 7.13 Some immunomodulators—the active agents of immunotherapy

Agent	Example
Interleukins	IL-2, IL-7, IL-12
Cytokines	Interferons, G-CSF, Imiquimod
Chemokines	CCL3, CCL26, CXCL7
Other	Cytosine phosphate-guanosine, oligodeoxynucleotides, glucans

to a fetus or to a newborn's body with witch's milk. In the case of the man-made passive immunization, antibodies are inserted into a body. Vaccines and detoxified bacterial products protect body for a long time, sometimes till one's dying day. The ready to use antibodies provide a temporary protection only; they have to be administering again in case of re-infection.

There are two ways of the man-made active immune booster, viz., (i) intake of the live but attenuated (weakened) microorganisms and (ii) intake of the killed microorganisms, their toxins or antigens. In both cases, a person gets vaccine or toxins, which do not cause a disease by themselves, but stimulate the immune system, making it able to recognize and to attack the particular microorganism. Generally, vaccines are administered parenterally (by injection) except live polio vaccine which is administered orally (by mouth). A possibility of using of the aerosols (when the vaccine gets into a body through the mucous membranes of a nose) is exploring for certain types of vaccines in addition to these two methods of vaccination.

The immune system is a complex and fantastic body system that protects people from bacteria, viruses, toxins, and other dangerous pathogens (germs). However, people often take their immune system for granted until something goes wrong with it. In the past, medical science primarily worked on ways of curing already-existing illness and disease, but today there is a growing emphasis on preventing illness by boosting the immune system's strength. Boosting body's immune system can help keep the body in peak condition to fight, repelling and destroying infections, viruses, and bacteria. There are many ways to help keep immune system functioning at its highest potential. If someone takes care of the immune system, immune system will, in turn, take care of him. Physical exercise keeps the body fit and active as well as helps the body to circulate lymph throughout the body. Simply, 30 min of moderate exercise a day can help the immune system to optimally carry out its defense. In addition, healthy and balanced diet, sufficient amounts of quality sleep, good hygiene habit, emotional health, healthy weight, practice prevention, etc., help to maintain sound health and immune system.

Natural immune system boosters and herbal antibiotics are known to prevent infections and illnesses. Synthetic and chemical agents may have adverse effects on the body. In this condition, herbal agents act fantabulously. Herbal agents are found to have zero side effects and show their action. Herbal immunity boosting agents are safe and effective. This phenomenon is present since ages, e.g., the concept of chyawanprash of Ayurvedic traditions developed >3000 years ago. Chyawanprash is a mixture of a large number of herbs (25–80) including amla and other ingredients and is claimed to possess antioxidants and immunomodulatory properties. There are many herbal immune boosters available in nature.

Natural remedies as immune system boosters

Natural and herbal remedies can provide much needed assistance in strengthening the immune system and getting the body's natural defense mechanisms in top shape. Holistic medicine recognizes that illness is caused primarily by weakened immune systems and not by pathogens. It is important to remember that

microorganisms like the flu virus, TB, etc., are around people all the time, but mostly they manage to resist becoming ill. Boosting the immune system naturally can allow the human body to fight off infectious agents without the drawbacks of conventional medication. There is a wide selection of medicinal herbs well known for their immune strengthening properties that are even safe for children too. Allowing the body to resolve infections without antibiotics will also help to strengthen the immune system against future attacks.

Black Elderberries (*Sambucus nigra*), a natural immune booster, are native to Europe and have a long history of use in herbal medicine. *Echinacea purpurea*, an American herb, has become famous for its antiviral, antifungal, and antibacterial properties. It is an excellent immune system tonic that boosts the body's immunity by stimulating the production of immune cells. *Astragalus membranaceus* has been used for centuries in traditional Chinese medicine to tone the immune system. *Astragalus* is an ideal remedy for anyone who is prone to recurrent infections such as the common cold, as it is able to increase the body's resistance and immune response to illness. *V. album* is commonly known to enhance the immune-stimulating properties of other ingredients, and it encourages repair of damaged cells. There are many herbs proven to improve immune function. Natural ingredients with immune boosting properties are *A. paniculata*, *Chondrus crispus* (Irish moss), *Crataegus monogyna* (Hawthorn), *C. oxycanthoides*, *Eleutherococcus senticosus* (Siberian ginseng), *Ficus carica*, *Glycyrrhiza glabra*, *H. officinalis*, *Hypoxis rooperi* (African star grass/wild potato), *Inula helenium*, *Mentha piperita*, *Olea europaea* (olive), *Panax ginseng* and other *Panax* spp., *P. emblica*, *Rosmarinus officinalis* (rosemary), *Schisandra chinensis*, *Solidago virgaurea*, *Thymus vulgaris* (thyme), *Verbascum thapsus* (mullein), *W. somnifera*, etc., act as excellent immune boosters. Over 30 species of medicinal mushrooms including *Auricularia auricula*, *A. polytricha*, *Agaricus bisporus* (Common mushroom), *A. blazei* (God's mushroom), *A. subrufescens*, *Agrocybe aegerita* (Chestnut mushroom), *Boletus edulis*, *Coprinus comatus*, *Cordyceps sinensis* (Caterpillar fungus), *Flammulina velutipes* (Enokitake), *Fomes fomentarius* (Tinder Conk mushroom), *G. lucidum* (Reishi), *Grifola frondosa* (Maitake), *Hericium erinaceus* (Lion's mane mushroom), *Hypsizygus tessellates*, *Inonotus obliquus* (Chaga Mushroom), *Lentinula edodes* (Shiitake), *Phallus indusiatus*, *Phellinus linteus* (Mesima), *Pleurotus citrinopileatus*, *P. djamor*, *P. ostreatus* (Oyster mushroom), *P. eryngii* (King Oyster mushroom), *Piptoporus betulinus*, *P. betulinus* (Birch bracket mushroom), *Schizophyllum commune* (Split-gill), *Sparassis crispa* (Cauliflower mushroom), *Tinder polypore*, *Trametes versicolor* (Turkey tail), *Tricholoma matsutake*, *Ustilago maydis*, and *Volvariella volvacea* are important for this purpose.

Medicinal mushrooms are used to treat and prevent a wide array of illnesses through their use as immune stimulants, immune modulators, adaptogens, and antioxidants. All of these natural medicines will go a long way in helping strengthen immune system against illness, disease, and infection. As immune stimulants, these natural products can be used to help treat cancer and fight infections by initiating an immune response which results in higher levels of white blood cells, cytokines, and antibodies and complement proteins. While not one of

the top immune-enhancing herbs, ginger (*Z. officinale*) does benefit the immune system. It has antibacterial, antiviral and antiparasitic activity. It has activity against certain types of cancer. It can stimulate phagocytosis and activate T-cells and modulate TH2.

Herbal immune boosting preparation

Herbal immune boosting preparation at home can be made with the following ingredients like 4 parts Echinacea root, 2 parts Thyme, 1 part Licorice and 1 part Elderberries. A cup of hot tea be prepared by adding 1 or 2 teaspoons of the premixed herbs to 1 cup of boiling water. Let it steep for 5–10 min, strain, and add honey (raw is best) to taste. To make a pitcher of tea for storing in the refrigerator, use 10 teaspoons or so of the premixed herbs for 8 cups of boiling water. Let it cool for a while and strain. Discard the used herbs, and put the tea in the refrigerator to drink over ice or reheated.

Biotechnology and high-tech herbal medicine

Biotechnology has applications in four major industrial areas, including health care (medical), crop production and agriculture, nonfood (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental uses. Biotechnology in healthcare sector along with the development of infrastructure, manpower, research, etc., includes development and manufacturing of biological reagents, biodiagnostics, biotherapeutics, preventive, therapeutic and, prophylactic vaccines, etc., and promoted biomedical innovation. The production of peptide hormones, new interferons and other lymphokines by the microbial and cell cultures, and new enzyme inhibitors of microbial origin are the most important for health care and pharmacy. Biotechnology is about to change health care and its delivery in profound ways; pharmacogenomics and the new genomic tools emerging from the biotechnology have revolutionized medicine and transformed the understanding of health and the provision of healthcare. Biotechnology has contributed to the discovery and manufacturing of traditional small molecule pharmaceutical drugs as well as drugs that are the product of biotechnology—biopharmaceutics. Examples of high-tech herbal medicine are plant-based vaccines, use of potato tubers as a biofactory for recombinant antibodies, etc.

Herbal vaccines

Vaccines help in stimulating the antibodies produced in human and animals and provide immune protection against several diseases. Plant-based vaccine production mainly involves the integration of transgene into the plant cells. Herbal or plant-based vaccine technologies involve the integration of the desired genes encoding the antigen protein for specific disease into the genome of plant tissues. The plants then start producing the exact protein that will be used for vaccinations. Agrobacterium-mediated gene transfer and transformation via genetically modified plant virus are the common methods that have been used to produce effective vaccines. New methods such as biolistic, electroporation, agroinfiltration, sonication, polyethylene glycol treatment, etc., have been developed to increase the efficiency of former methods. The flexibility of the plant expressed vaccine system,

combined with its low cost and ability to massively scale may provide vaccine protection not only to citizens of the developed as well as developing countries of the world that cannot currently afford vaccines. Other uses of plant-expressed vaccines including the successful creation of edible bananas that protect against the Norwalk virus. Table 7.14 showing examples of some plant-based vaccines for human and animal diseases.

Prevention is better than cure as the proverb implies that it is unwise to become diseased and go under treatment to get rid of the curse of the imposed disease rather the disease should be prevented before the attack. It is always safe to prevent the enemy before the attack. Boosting the immune system and prohibiting the entry of microorganisms is better than acting upon them later. Immunotherapy is a medical term defined as the treatment of disease by inducing, enhancing, or suppressing an immune response. Immunotherapy designed to elicit or amplify an immune response are classified as activation immunotherapy, while immunotherapy that reduce or suppress are classified as suppression immunotherapy.

Activation immunotherapies—cancer immunotherapy

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. Dr. William Coley used Coley's Toxins in the late 1800s as crude immunotherapy with some success. Immuno-cell therapy for cancer was first introduced by Rosenberg and his colleagues of National Institute of Health, USA. In the late 80s, they published an article in which they reported a low tumor regression rate (2.6–3.3%) in 1205 patients with metastatic cancer who underwent different types of active specific immunotherapy (ASI), and suggested that immuno-cell therapy along with specific chemotherapy is the future of cancer immunotherapy. Initially, immunotherapy treatments involved administration of cytokines such as interleukin. Thereafter, the adverse effects of such intravenously administered cytokines lead to the extraction of the lymphocytes from the blood and expanding in vitro against tumor antigen before injecting the cells with appropriate stimulatory cytokines. The cells will then specifically target and destroy the tumor expressing antigen against which they have been raised.

The concept of this treatment started in the US in the 80s and fully fledged clinical treatments on a routine basis have been in practice in Japan since 1990. Randomized controlled studies in different cancers resulting in significant increase in survival and disease-free period have been reported and its efficacy is enhanced by 20–30% when cell-based immunotherapy is combined with other conventional treatment methods. BCG immunotherapy for early stage (noninvasive) bladder cancer utilizes *instillation* of attenuated live bacteria into the bladder, and is effective in preventing recurrence in up to two-thirds of cases. Topical immunotherapy utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patients own killer T-cells to destroy warts, actinic keratoses, basal cell cancer, vaginal intraepithelial neoplasia, squamous cell cancer, cutaneous lymphoma, and superficial malignant melanoma. Injection immunotherapy uses mumps, candida the HPV vaccine or trichophytin antigen

Table 7.14 The plant-based vaccines production for human and animal diseases

Disease	Pathogens	Plants	Transformation method	References
(i) Avian H5N1 influenza	Hemagglutinin protein of H5N1	<i>Nicotiana benthamiana</i>	Agrobacterium	Greer (2015)
(ii) Bluetongue	Bluetongue virus	<i>Nicotiana benthamiana</i>	Agroinfiltration	Thuenemann et al. (2013)
(iii) Dengue	Dengue virus type 2 E glycoprotein (EIII)	<i>Nicotiana tabacum</i> cv. MD609	Agrobacterium tumefaciens	Kim et al. (2009)
(iv) Diabetics	Insulin	Safflower	Agrobacterium tumefaciens	Penney et al. (2011)
(v) Diarrheal	Norwalk virus	<i>Nicotiana benthamiana</i>	Agrobacterium tumefaciens	Lai and Chen (2012)
(vi) Diarrheal	Enterotoxigenic <i>Escherichia coli</i>	Corn	–	Tacket et al. (2004)
(vii) Ebola	Ebola virus	<i>Nicotiana benthamiana</i>	Agroinfiltration	Phoolcharoen et al. (2011)
(viii) Foot-and-mouth disease	Foot-and-mouth disease virus	<i>Stylosanthes guianensis</i> cv. Reyan II		Wang et al. (2008)
(ix) Gaucher disease	Taliglucerase alfa	Carrot	Stable transformation	Malabadi et al. (2012)
(x) Hepatitis B	Hepatitis B surface antigen	Tomato	Agrobacterium tumefaciens	Li et al. (2011)
(xi) Human immunodeficiency	HIV	Tobacco	Agroinfiltration	Strasser et al. (2009)
(xii) Nerve agents attack	Acetylcholinesterase	Tobacco	PEGylated	Atsmon et al. (2015)
(xiii) Rabies	Rabies virus	<i>Nicotiana benthamiana</i> , tomato	Agroinfiltration	Perea Arango et al. (2008)
Tuberculosis	<i>Mycobacterium tuberculosis</i>	<i>Arabidopsis thaliana</i>	Agrobacterium	Rigano et al. (2004)

injections to treat warts (HPV induced tumors). Lung cancer has been demonstrated to potentially respond to immunotherapy.

Dendritic cell-based immunotherapy

Dendritic cells (DCs) are professional antigen-presenting cells (APC). They are found in most tissues of the body and are particularly abundant in those that are interfaces between the external and internal environments (e.g., skin, lungs, mucosa, and lymphoid tissues and in the lining of the gastrointestinal tract). DCs get their name from their surface projections that resemble the dendrites of neurons. Their main function is to process antigens and present them to T cells to promote immunity to foreign antigens and tolerance to self-antigens. They also secrete cytokines to regulate immune responses. Once activated, they migrate to the lymph nodes where they interact with T-cells and B-cells to initiate and shape the adaptive immune response. They act as messengers between the innate and the adaptive immune systems.

Dendritic cells can be stimulated to activate a cytotoxic response towards an antigen. Dendritic cells, a type of antigen-presenting cell, are harvested from a patient. These cells are then either pulsed with an antigen or transfected with a viral vector. Upon transfusion back into the patient, these activated cells present tumor antigen to effector lymphocytes (CD4 + T cells, CD8 + T cells, and B cells). This initiates a cytotoxic response to occur against cells expressing tumor antigens (against which the adaptive response has now been primed). The cancer vaccine Sipuleucel-T is one example of this approach. DC-based vaccinations represent a promising approach for the immunotherapy of cancer and infectious diseases as DCs play an essential role in initiating cellular immune responses.

T-cell adoptive transfer

Adoptive cell transfer uses T-cell-based cytotoxic responses to attack cancer cells. T-cells that have a natural or genetically engineered reactivity to a patient's cancer are generated in vitro and then transferred back into the cancer patient. One study using autologous tumor-infiltrating lymphocytes was an effective treatment for patients with metastatic melanoma. This can be achieved by taking T-cells that are found in the tumor of the patient, which are trained to attack the cancerous cells. These T-cells are referred to as tumor-infiltrating lymphocytes (TIL) and are then encouraged to multiply in vitro using high concentrations of IL-2, anti-CD3, and allo-reactive feeder cells. These T-cells are then transferred back into the patient along with exogenous administration of IL-2 to further boost their anticancer activity.

Thus far, a 51% objective response rate has been observed; and in some patients, tumors shrank to undetectable size.

The initial studies of adoptive cell transfer using TIL, however, revealed that persistence of the transferred cells in vivo was too short. Before reinfusion, lymphodepletion of the recipient is required to eliminate regulatory T-cells as well as normal endogenous lymphocytes that compete with the transferred cells for homeostatic cytokines. Lymphodepletion was made by total body irradiation prior to transfer of the expanded TIL. The trend for increasing survival as a function of

increasing lymphodepletion was highly significant ($P = 0.007$). Transferred cells expanded *in vivo* and persisted in the peripheral blood in many patients, sometimes achieving levels of 75% of all CD8⁺ T-cells at 6–12 months after infusion. Clinical trials based on adoptive cell transfer of TILs for patients with metastatic melanoma are currently ongoing at the National Cancer Institute (Bethesda, MD, USA), Moffitt Cancer Center (Tampa, FL, USA), MD Anderson Cancer Center (Houston, TX, USA), Sheba Medical Center (Tel Hashomer, Israel), Herlev University Hospital (Herlev, Denmark) and NKI Antonie van Leeuwenhoek (Amsterdam, Netherlands).

Autologous immune enhancement therapy

The autologous immune enhancement therapy (AIET) is an autologous immune cell-based therapy, wherein the patient's own peripheral blood-derived NK cells Cytotoxic T-lymphocytes and other relevant immune cells are expanded *in vitro* and then re-infused to tackle cancer. There are also studies proving their efficacy against hepatitis C viral infection, chronic fatigue syndrome, and HHV6 infection.

Genetically engineered T-cells

Genetically engineered T-cells are created by infecting patient's cells with a virus that contains a copy of a T cell receptor (TCR) gene that is specialized to recognize tumor antigens. The virus is not able to reproduce within the cell, however, integrates into the human genome. This is beneficial as new TCR gene remains stable in the T-cell. A patient's own T-cells are exposed to these viruses and then expanded nonspecifically or stimulated using the genetically engineered TCR. The cells are then transferred back into the patient and ready to have an immune response against tumor. Morgan et al. (2006) demonstrated that the adoptive cell transfer of lymphocytes transduced with retrovirus encoding TCRs that recognize a cancer antigen are able to mediate antitumor responses in patients with metastatic melanomas. This therapy has been demonstrated to result in objective clinical responses in patients with refractory stage IV cancer. The Surgery Branch of the National Cancer Institute (Bethesda, Maryland) is actively investigating this form of cancer treatment for patients suffering aggressive melanomas. The use of adoptive cell transfer with genetically engineered T-cells is a promising new approach to the treatment of a variety of cancers.

In one case study, United States doctors from the Clinical Research Division, led by Dr. Cassian Yee at Fred Hutchinson Cancer Research Center in Seattle had successfully treated a patient with advanced skin cancer by injecting the patient with immune cells cloned from his own immune system. The patient was free from tumours within 8 weeks of treatment. Dr. Cassian Yee described the research findings at The Cancer Research Institute International 2008 Symposia Series. Responses, however, were not seen in other patients in this clinical trial. Larger trials are now under way.

Immune recovery

The potential use of immunotherapy is known to restore the immune system of patients with immune deficiencies as result of infection or chemotherapy. For

example, cytokines have been tested in clinical trials interleukin-7 has been in clinical trials for HIV and cancer patients. In addition, interleukin-2 has also been tested in HIV patients.

Vaccination

Antimicrobial immunotherapy, which includes vaccination, involves activating the immune system to respond to an infectious agent.

Suppression immunotherapies

Immune suppression dampens on abnormal immune response in autoimmune diseases or reduces a normal immune response to prevent rejection of transplanted organs or cells.

Immunosuppressive drugs

Immunosuppressive drugs are important tools in the management of organ transplantation and autoimmune disease. Immune responses depend on lymphocyte proliferation, and cytostatic drugs are immunosuppressive. Glucocorticoids are somewhat more specific inhibitors of lymphocyte activation, whereas inhibitors of immunophilins more specifically target T lymphocyte activation. Immunosuppressive antibodies target an increasingly broad array of steps in the immune response, and there are still other drugs that modulate immune responses.

Immune tolerance

Immune tolerance is the process by which the body naturally does not launch an immune system attack on its own tissues. An immune tolerance therapy seeks to reset the immune system so that the body stops mistakenly attacking its own organs or cells in autoimmune disease or accepts foreign tissue in organ transplantation. A brief treatment should then reduce or eliminate the need for lifelong immunosuppression and the chances of attendant side effects, in the case of transplantation, or preserve the body's own function, at least in part, in cases of type 1 diabetes or other autoimmune disorders.

Allergies

Immunotherapy is also used to treat allergies. While other allergy treatments (such as antihistamines or corticosteroids) treat only the symptoms of allergic disease, immunotherapy is the only available treatment that can modify the natural course of the allergic disease, by reducing sensitivity to allergens.

A 1-to-5-year individually tailored regimen of injections may result in long-term benefits. Recent research suggests that patients who complete immunotherapy may continue to see benefits for years to come. Immunotherapy does not work for everyone and is only partly effective in some people, but it offers allergy sufferers the chance to eventually reduce or stop symptomatic/rescue medication.

The therapy is indicated for people who are extremely allergic or who cannot avoid specific allergens. For example, they may not be able to live a normal life and completely avoid pollen, dust, mites, mold spores, pet dander, insect venom, and certain other common triggers of allergic reactions. Immunotherapy is generally not indicated for food or medicinal allergies. Immunotherapy is typically individually

tailored and administered by an allergist (allergologist) or through specialized physician offices. Injection schedules are available in some healthcare systems and can be prescribed by family physicians. This therapy is particularly useful for people with allergic rhinitis or asthma.

The therapy is particularly likely to be successful if it begins early in life or soon after the allergy develops for the first time. In the past, this was called a serum, but this is an incorrect name. Most allergists now call this mixture an allergy extract. The first shots contain very tiny amounts of the allergen or antigen to which one is allergic. With progressively increasing dosages over time, one's body adjusts to the allergen and becomes less sensitive to it, in a process known as desensitization. A recently approved sublingual tablet (Grazax), containing a grass pollen extract, is similarly effective with few side effects, and can be self-administered at home, including by those patients who also suffer from allergic asthma, a condition which precludes the use of injection-based desensitization. To read more about this topic, see allergy and hyposensitization.

Helminthic therapies

Recent research into the clinical effectiveness of Whipworm ova (*Trichuris suis*) and Hookworm (*Necator americanus*) for the treatment of certain immunological diseases and allergies means that these organisms must be classified as immunotherapeutic agents. Helminthic therapy is being investigated as a potentially highly effective treatment for the symptoms and or disease process in disorders such as relapsing-remitting multiple sclerosis Crohn's, allergies and asthma. The precise mechanism of how the helminths modulate the immune response, ensuring their survival in the host and incidentally effectively modulating autoimmune disease processes, is currently unknown. However, several broad mechanisms have been postulated, such as a re-polarization of the Th1/Th2 response, and modulation of dendritic cell function. The helminths downregulate the pro-inflammatory Th1 cytokines, Interleukin-12 (IL-12), Interferon Gamma (IFN- γ) and Tumour Necrosis Factor-Alpha (TNF- α), while promoting the production of regulatory Th2 cytokines such as IL-10, IL-4, IL-5, and IL-13.

That helminths modulate host immune response is proven, as the core assertion of the hygiene hypothesis appears to have been, with the recent publication of a study demonstrating that co-evolution with helminths has shaped at least some of the genes associated with Interleukin expression and immunological disorders, like Crohn's, ulcerative colitis and Celiac Disease. Much of the research that has been published now indicates a key role, for what has been traditionally regarded as disease-causing organisms, so that their relationship to humans as hosts should not be classified as parasitic, rather as mutualistic, symbionts.

7.5 Biotechnology of Disease Prevention

Most developments in biotechnology originated for their potential applications in health care of both human and animal. And it is in this sector that the contributions of biotechnology are more frequent, more notable and more rewarding (both financially and psychologically). It is difficult to summarize the whole gamut of contributions in a text of limited space; these could be grouped under the following broad heads: (i) disease prevention, (ii) disease detection, (iii) therapeutic agents, (iv) correction of genetic diseases, (v) fertility control, and (vi) forensic medicine. It is aimed to highlight the major developments under each of these categories by citing appropriate examples.

Disease prevention (Vaccines)

Prevention of diseases is the most desirable, most convenient and highly effective approach to health, this is achieved by vaccination or immunization using biological preparations called vaccines. Vaccines represent an invaluable contribution of biotechnology as they provide protection against even such diseases for which effective cures are not yet available. The effectiveness of vaccines may be appreciated from the fact that smallpox, once a dreaded disease the world over, has been completely eradicated from the world; the last case of smallpox was reported in 1977. The various vaccines can be grouped under the following types: (i) conventional vaccines (live vaccines, inactivated pathogens), (ii) purified antigen vaccines, and (iii) recombinant vaccines (recombinant proteins/polypeptides, DNA vaccines) (Fig. 7.8).

An ideal vaccine

An ideal vaccine or vaccination protocol should have the following features:

- (i) It should not be tumorigenic or toxic or pathogenic, i.e., it should be safe.
- (ii) It should have very low levels of side effects in normal individuals.
- (iii) It should not cause problems in individuals with the impaired immune system.
- (iv) It should not spread either within the vaccinated individual or to other individuals (live vaccines).
- (v) It should not contaminate the environment.
- (vi) It should be effective in producing long-lasting humoral and cellular immunities.

Conventional vaccines

Conventional vaccines consist of whole pathogenic organisms which may either be killed (most bacterial vaccines and some viral vaccines), or live vaccines where the virulence of pathogens is greatly reduced or attenuated (most viral vaccines). Conventional vaccines, although highly effective and relatively easy to produce at low cost, suffer from the following limitations:

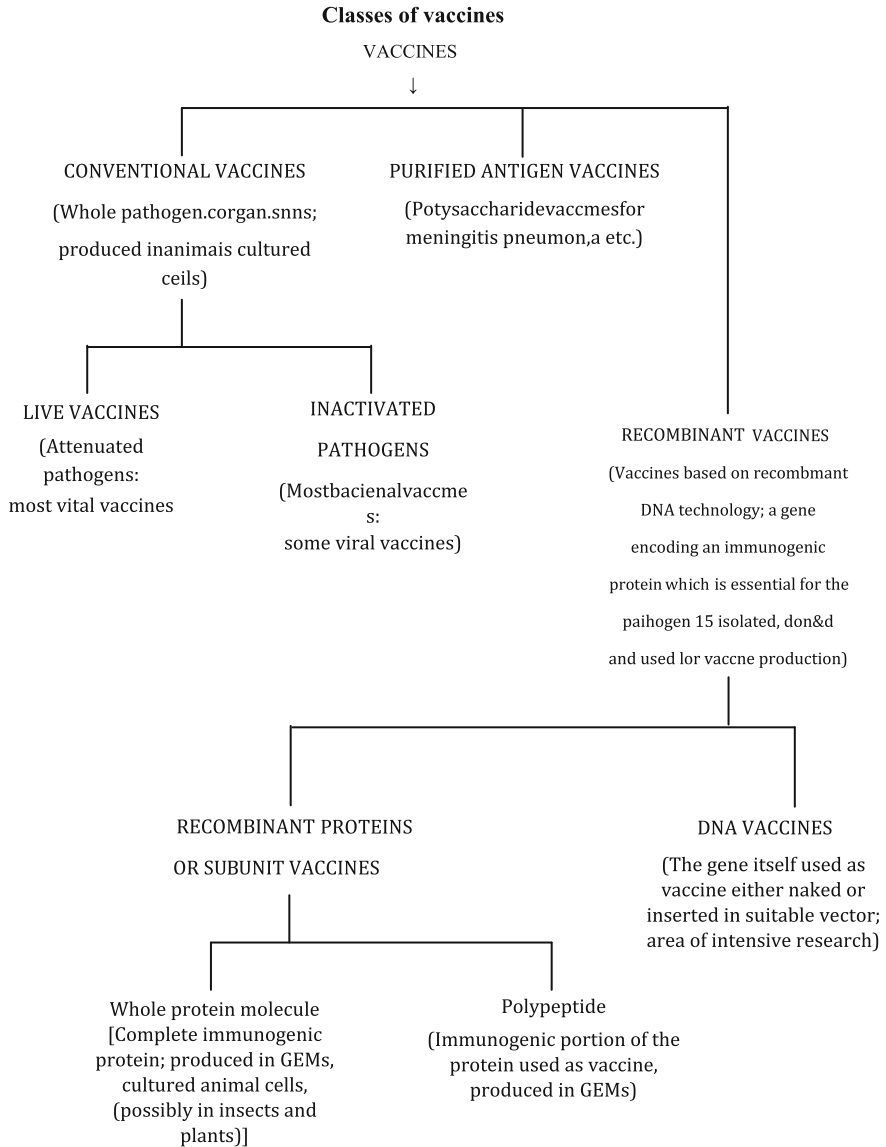


Fig. 7.8 Classes of different vaccines either in commercial use or in various stages of development (DNA vaccines)

- (i) in many cases, live vaccines have to be used since killed pathogen vaccines are ineffective;
- (ii) live vaccines are generally based on cultured animal cells, hence expensive tissue culture setup is essential;
- (iii) live vaccines are heat-labile due to the pathogen inactivation by heat;

- (iv) conventional vaccines carry a variable risk of disease development due to the occasional presence of active virus particles (in case of inactivated vaccines; e.g., outbreak of foot-and-mouth disease in Europe), or reversion to virulence after replication in the vaccinated individuals (in case of attenuated live vaccines; 1 or 2×10^{-6} cases in case of live polio vaccine); and
- (v) in many cases, they are difficult to produce, e.g., hepatitis b virus does not grow in high titer in cultured cells;

These limitations have prompted the successful development of vaccines based on purified antigens and of recombinant vaccines, which are rather costly, at least for the present.

Purified antigen vaccines

These vaccines are based on purified antigens isolated from the concerned pathogens, i.e., these are nonrecombinant). Since they do not contain the organism, the risk of pathogenicity is avoided. However, (their cost is higher due to the steps involved in purification and vaccine preparation, and many of the isolated antigens are poorly immunogenic. Successful examples of such vaccines are mostly from bacteria, e.g., vaccines based on polysaccharide antigens from the bacterial cell wall capsules of *Neisseria meningitidis* (causing meningitis) and *Streptococcus pneumoniae* (causing pneumonia).

Many bacteria produce exotoxins which are highly immunogenic. But these toxins produce toxic effects, the intensity of which decreases with storage and this—decline is accelerated by heat, formaldehyde, and other chemicals. Fortunately, many exotoxins that have lost their toxicity retain their immunogenicity. They are called toxoids and are used as effective vaccines, e.g., toxoids of the pathogens causing tetanus, diphtheria, gangrene, etc. Precipitation of toxoids with alum enhances their immunogenicity. The toxoid vaccines are quite effective and cheap.

Some toxoids are very good adjuvants, i.e., increase the immunogenicity of other antigens, e.g., diphtheria toxoid. For example, the B polysaccharide of *Haemophilus influenzae* is poorly immunogenic. But when the B polysaccharide is combined with diphtheria toxoid. Its immunogenicity is greatly increased. In many cases, such adjuvant activities can be used to great advantage since most of the isolated antigens from pathogens are poorly immunogenic.

Recombinant vaccines

A recombinant vaccine contains either a protein or a gene encoding a protein of a pathogen origin that is immunogenic and critical to the pathogen function; the vaccine is produced using recombinant DNA technology. The vaccines based on recombinant proteins (=proteins produced by recombinant DNA technology) are also called subunit vaccines. The logic of such vaccines, in simple terms, is as follows. Proteins are generally immunogenic, and many of them are critical for the pathogenic organism. The genes encoding such proteins can be identified and isolated from a pathogen and expressed in *E. coli* or some other suitable host for a mass production of the proteins.

The concerned proteins are then purified and mixed with suitable stabilizers and adjuvants, if required, and used for immunization.

The different steps involved in the development of a recombinant protein-based vaccine may be simply summarized as follows: (i) The first step is to identify a protein that is both immunogenic and critical for the pathogen. (ii) The gene encoding this protein is then identified and isolated. (iii) The gene is integrated into a suitable expression vector and introduced into a suitable host where it expresses the protein in large quantities. (iv) The protein is then isolated and purified from the host cells, and (v) It is used for the preparation of vaccine. The host organisms used for expression of immunogenic proteins to be used as vaccines may be any one of the following:

- (i) A genetically engineered microorganism, e.g., yeast for the expression of hepatitis B surface antigen (HBsAg) used as vaccine against hepatitis B virus (this vaccine is approved for marketing in India) (Table 7.15).

Table 7.15 Products generated from genetically engineered microbes (GEMS). The products are either in current therapeutic use or in advanced stages of development

Product	GEM	Application
Currently in therapeutic use insulin ^a	<i>E. coli</i> and yeast	Diabetes
Human growth hormone	<i>E. coli</i>	Dwarfism
Interferons ^a	<i>E. coli</i>	Viral diseases, cancer, AIDS
Interleukins	<i>E. coli</i>	Various cancers
Hepatitis B surface antigen ^a	Yeast	Vaccine against hepatitis B
Streptokinase	<i>E. coli</i>	Thrombolysis
Epidermal growth factor	<i>E. coli</i> Yeast	Wound and burn healing
Granulocyte macrophage colony-stimulating factor ^a	Yeast	Cancer, AIDS
Granulocyte colony-stimulating factor ^a	<i>E. coli</i>	Cancer, AIDS, bone marrow transplantation
Bovine growth hormone	<i>E. coli</i>	Increased milkyield
Tumour necrosis factor		Sepsis, cancer
In advanced stages of development		
Atrial natriuretic factor (ANF)	Yeast	Hypertension and kidney diseases Thrombolysis
Plasminogen activator (including chimacrics and modifications)	<i>E. coli</i>	Cardiac treatment and organ transplants
Superoxide dismutase	<i>E. coli</i>	Antitumour and antiviral therapy Thrombolysis
Urokinase	<i>E. coli</i>	Neuropathic ulcers
Fibroblast growth factor	<i>E. coli</i> and Yeast	Osteoporosis, metabolism
Insulin like growth factor	<i>E. coli</i> and Yeast	Peripheral neuropathies Diabetic ulcers

(continued)

Table 7.15 (continued)

Product	GEM	Application
Nerve growth factor	<i>E. coli</i> and Yeast	Blood substitute
Platelet derived growth factor	<i>E. coli</i> Yeast	Cystic fibrosis
Haemoglobin (adult)	Yeast	Viral infection
Thymosin α 1	<i>E. coli</i>	

^aApproved for marketing in India. Source Ghosh (1995)

- (ii) Cultured animal cells, e.g., HBsAg expressed in CHO (Chinese hamster ovary Chinese hamster ovary) cell line and C-127 cell line; the vaccine is in advanced stages of development.
- (iii) Transgenic plants, e.g., HBsAg, HIV-1 (human immunodeficiency virus I) epitope. Rhinovirus 14 epitope (the last two for use as vaccines against HIV-1; in experimental stages).
- (iv) Insect larvae; the gene is integrated into a baculovirus (DNA viruses) which is used to infect insect larvae. Often, a very high quantity of the recombinant protein is produced. For example, up to 68% of the total protein of *Spodoptera exigua* larvae infected with a recombinant baculovirus is the recombinant protein. This protein can be purified for use as vaccine or may be used in diagnostic tests without purification. This approach has also been used to produce monoclonal antibodies to uncommon antigens. e.g., Alzheimer's protein.

So far a large number of recombinant immunogenic proteins of pathogens have been produced and evaluated. In general, a majority of such proteins are ineffective or only poorly effective in immunization. In some cases, at least, this problem may be due to the essential requirement for the immunogenic protein to be present in a specific aggregate form, e.g., HBsAg can be used for effective immunization only when it forms virus-like particles (of about 22 nm). Thus far hepatitis B vaccine is the only good example of a recombinant protein vaccine. An antimalarial vaccine based on the recombinant circumsporozoite protein of the sporozoite stage of the parasite (*Plasmodium falciparum*) is in advanced stages of development.

Recombinant polypeptide vaccines

Generally, the whole protein molecule is not necessary for immunogenicity; the immunogenic property is usually confined to a small portion of the protein molecule. For example, the immunogenicity of foot-and-mouth disease virus coat protein is due to its amino acids 114–160, and also 201–213. Segments of proteins containing either of these two amino acid sequences are effective in immunization; they induce antibodies which neutralize the virus and thereby provide protection against the foot-and-mouth disease. Similarly, the immunogenicity of the coat protein of feline leukemia virus (FLV) is due to a 14-amino acid long segment; this segment produced a partial immunogenic response in guinea pigs. Table 7.15 shows

products generated from genetically engineered microbes (GEMS). The products are either in current therapeutic use or in advanced stages of development.

In some cases, the immunogenic protein may be composed of two or more distinct polypeptides. In such cases, it may be desirable to use only one of the polypeptides as a vaccine for various reasons. For example, the cholera enterotoxin (produced by *Vibrio cholerae*) consists of 3 polypeptides, viz., A₁, A₂, and B polypeptides. The A polypeptides are toxic, while the B polypeptide is nontoxic but immunogenic. The gene encoding B polypeptide has been cloned, and the recombinant B polypeptide thus produced is being used as a vaccine against cholera; the recombinant B polypeptide is used, in combination with inactivated cholera cells, as an oral vaccine in place of the conventional injectable vaccine.

Recombinant protein or polypeptide vaccines are very safe since whole organisms are not involved. They are also of high efficacy. But (i) their cost is very high and often prohibitive, since they are produced by either bacterial, fermentation, or in animal cell cultures. (ii) They have to be stored at low temperatures since heat destabilizes the proteins, and (iii) This makes their storage and transportation, especially in developing countries, problematic and often limiting.

DNA vaccines

Recently, vaccines based on DNA are being developed, and the results obtained with influenza virus (advanced stages of testing) are quite exciting: these are regarded as the third revolution in vaccines. The strategy of DNA vaccines is as follows. The gene encoding the relevant immunogenic protein is isolated, cloned and then integrated into a suitable expression vector. This preparation is introduced into the individual to be immunized. The gene is ultimately expressed in the vaccinated individual and the immunogenic protein is expressed in sufficient quantities to invoke both humoral and cell-mediated immunities. It may be pointed out that cell-mediated immune response is essential for recovery from infectious diseases. The various approaches for DNA vaccines are as follows: (i) injection of pure DNA (or RNA) preparation into muscle; (ii) use of vectors (e.g., vaccinia viruses, adenoviruses, retroviruses, *E. coli*, *Salmonella typhimurium*, herpes viruses, etc.) for delivery of the gene, (iii) reimplantation of autologous cells (cells of the individual to be vaccinated) into which the gene has been transferred, and (iv) particle gun delivery of plasmid DNA which contains the gene in an expression cassette.

Injection of pure DNA or RNA into the skeletal muscle leads to the uptake and expression of the DNA in the muscle cells. When a gene encoding an immunogenic protein is so introduced, its expression also results in immunization of the individuals. This approach has potential for delivery of DNA vaccines. The DNA most likely enters the skeletal muscle cells through transient discontinuities in their plasma lemma produced by stretching of the muscle cells during exercise.

Another approach is to remove cells from the body of an individual into which the concerned immunogen encoding gene is introduced and expressed. These cells are then reintroduced into the body of the individual in a variety of ways, e.g., simple infusion, implantation, encapsulation, etc. This approach, although more cumbersome, has the advantage of enabling control of the modified cells within containment.

The immunogen encoding gene may be integrated into an expression plasmid, which is purified, coated on gold or tungsten particles and introduced into skin cells by a particle gun. Antigen-encoding genes introduced into the skin of mice and guinea pigs elicited humoral immune response; it is not known if cellular immunity is also induced. Plasmid DNA is noninfectious, heat stable and offers other advantages over viral/bacterial vectors. The skin cells are usually shed off in a few days after the inoculation so that there is no long-term persistence of the modified cells.

The approach holding considerable promise employs a live vector for the delivery of immunogen-encoding gene into the vaccinated individuals. The most advanced and promising vectors are: vaccinia viruses, adenoviruses, *E. coli*, *S. typhimurium*, other poxviruses, herpesviruses etc. The concerned gene is introduced into the genome of selected viral/bacterial vector which is suitably attenuated, and the live microorganisms are used for vaccination. Of the various vectors studied, vaccinia virus appears to be the most promising.

Vaccinia virus (W) is a close relative of the variola virus causing smallpox and was used as the vaccine to generate protection against smallpox. This virus has many useful features including stability in freeze-dried preparations, low production cost, and simple administration through ruptured skin cells. VV offers 19 possible sites for integration and expression of foreign genes. Generally, antigen encoding genes are inserted within its thymidine kinase (TK) locus which makes the virus TK and attenuates its pathogenicity. Further attenuation of VV can be achieved by integration, in its genome, of lymphokine genes like interferon gamma (IFN- γ) or interleukin-2 (IL-2). IFN- γ and IL-2 have, in addition, adjuvant activity and promote the immunogenicity of the introduced antigen to a level comparable to complete Freund's adjuvant.

A large number of genes encoding antigenic proteins have been integrated into the W genome which was then used for vaccination. The antigens included viral proteins like rabies virus glycoprotein, herpes simplex virus glycoprotein D, hepatitis B surface antigen, vesicular stomatitis glycoprotein etc. These recombinant vaccinia viruses induced both humoral and cellular immunity, and protected the immunized animals from the concerned viruses. Recently, a highly effective vaccine against rinderpest virus has been developed by inserting the viral genes H and F in the VV genes TK and HA. Cattle immunized with the recombinant VV vaccine were completely protected even when they were challenged by a more than 1000 times the normally lethal inoculum. The recombinant VV vaccine against rabies is also highly effective. Some antigens of HIV have been produced by recombinant VV in experimental animals and human volunteers; these antigens induced detectable cellular immunity to the expressed HIV protein. A recombinant VV containing the chimeric gene for IFN-g and the structural proteins of HIV-1 is a quite promising candidate for a safe vaccine against HIV-1.

Several antigen encoding genes, each from a different pathogen, may be incorporated into a single VV genome. Such a recombinant VV vaccine will produce immunity to several diseases from a single inoculation; such vaccines are

called polyvalent vaccines. There is little data on the efficiency of such vaccines. The use of DNA for immunization is often called genetic immunization.

Recombinant VV (i) is not transmitted from vaccinated to contact animals. (ii) and induces both humoral and cellular immune responses. But (i) individuals previously immunized or exposed to infection by VV may respond poorly to recombinant VV vaccines. In addition, (ii) children and adults with congenital or acquired immunodeficiency may run the risk of severe infections; this could be resolved by incorporating the VV genome the gene IL-2 or IFN- γ .

DNA vaccines offer the following advantages: (i) purification and preparation of DNA for vaccines is easier, cheaper and more rapid, (ii) they are safer and more specific because of high purity, and (iii) they elicit a more potent immune response than purified protein vaccines.

Disease diagnosis

An accurate diagnosis of the disease and its causal organism is critical to its effective management and cure. Conventionally, disease diagnosis is based on the following.

- (i) Microscopy. The specimen (tissue, body fluid, excreta, pus, exudates, etc.) are subjected to microscopic examination for detection of the causal organisms, e.g., stool examination for ova and cyst.
- (ii) Culture of the specimen on specific and selective media to allow specific pathogens to grow, which are then tested for their susceptibility to various therapeutic agents, e.g., antibiotics.
- (iii) Detection and measurement of the pathogen-specific antibodies produced by the patient in response to the invasion by pathogen, e.g., in case of viral infections.

These tests are often tedious, take a long time (e.g., culture methods), may yield ambiguous results (e.g., immunological assays since they are based on polyclonal antibodies), and some of them cannot be applied in certain cases (e.g., antibody titer estimation in case of latent viral infections). Novel diagnostic approaches have been developed by biotechnology which are precise and very rapid, viz., (i) probes and (ii) monoclonal antibodies.

Probes

Probes are small (15–30 bases long) nucleotide (DNA/RNA) sequences used to detect the presence of complementary sequences in nucleic acid samples. Both DNA and RNA are used as probes. The probes can be prepared in many ways, and are either radioactively or non-radioactively labeled. Probes are being used in clinical diagnosis for the detection of microorganisms in various samples, e.g., tissues, excreta, body fluids, etc. Use of probes for disease diagnosis offers several advantages over the conventional diagnostic tools which are briefly listed below.

- (i) They are highly specific, relatively rapid, and much simpler.
- (ii) They are extremely powerful especially when combined with PCR; even a single molecule in the test sample can be detected.
- (iii) Since the culture of microbes is not required, the risk of accidental infection to laboratory personnel is eliminated and considerable time is saved.
- (iv) It is applicable to even such organisms which cannot be cultured.
- (v) Probes detect even latent viral infections which do not lead to an increase in antibody titer in the blood.
- (vi) A single species-specific probe can identify all the serotypes of a pathogen (Each of them may require a separate antibody).
- (vii) Pure probe preparations are relatively easily obtained.

However, since probes are usually radioactively labeled, they present a health hazard in handling and disposal. Therefore, the emphasis is shifting to non-radioactively labeled probes.

Probes are available for the detection of a variety of pathogenic microorganisms (Table 7.16). Probes can be used as follows: (i) hybridization (dot blot. Southern, in situ) and (ii) ligase chain reaction (LCR).

Hybridization—DNA may be isolated from the test samples and subjected to Southern blot or dot blot hybridization with the probe. For dot blot analyses, test samples like blood are generally lysed directly on the nitrocellulose filter. A probe can hybridize with a test.

DNA sample only when the latter contains the complementary sequence. The probes used in diagnostic assays are highly specific to the concerned pathogenic microorganisms. Therefore, a positive hybridization signal of a test DNA sample with a given probe reveals the presence of concerned microorganism.

Probes are used for hybridization assays using microscopic preparations of tissues. Generally, the tissues are fixed in formalin, embedded in paraffin, sectioned and stained with conventional stains like eosin and hematoxylin for routine examination. Subsequently, probes are used for in situ hybridization to detect the presence of concerned pathogens. This approach has proved quite useful for the detection of viral pathogens. The cytopathological data obtained by routine microscopic observations can then be correlated with the presence of specific pathogens to obtain a greater insight.

Table 7.16 Some selected microorganisms against which probes are available

Protozoa	Helminths	Bacteria	Viruses
Leishmania (Kala-azar)	Schistosomes (human blood flukes)	Legionella <i>Mycobacterium tuberculosis</i> complex	Herpes virus type 2
Trypanosoma (Sleeping sickness)	Wuchereria and Brugia (Filaria)	<i>Mycobacterium</i> spp. <i>Mycobacterium avium</i> - <i>intracellulare</i>	Herpes virus type 1 and 2
Plasmodium (Malaria)	Onchocerca (River blindness) Trichinella Taenia solium	<i>Mycoplasma pneumoniae</i> <i>Chlamydia campylobacter</i> spp.	

Ligase chain reaction

In ligase chain reaction, (i) the clinical sample is prepared according to a protocol which liberates the DNA present in the sample. (ii) The prepared clinical sample is added to a reaction mixture containing thermostable DNA ligase, a vast excess of two double-strand oligonucleotide probes specific to the pathogen to be detected, and NAD (nicotinamide adenine dinucleotide). The two probes are blunt-ended, represent contiguous segments of the pathogen genome, and each of them is 15–30 bases long, therefore the target sequence is 35–60 bases long. The target sequence must be specific to the pathogen to be detected. (iii) The reaction mixture is heated in a thermocycler (also used for PCR) to 94 °C to ensure strand separation of both the target DNA and the probes; (iv) The temperature is then lowered to 55 °C to allow the probes to pair with the target DNA. Now, ligase joins the adjacent 3'-OH of one strand of probe 1 to the 5' phosphate of probe 2 strand; the complementary strands of the two probes are also similarly joined. The product of ligation of the two probes is called amplicon.

The second cycle of the ligase chain reaction (LCR) is initiated by heating the reaction mixture to 94 °C. In this and subsequent cycles of LCR, both the target DNA and the amplicons serve as targets for probes and, as a result, for amplification; this leads to an exponential amplification of the amplicons. The amplicons are detected by gel electrophoresis of the reaction mixture, ethidium bromide staining, and viewing under UV light. There will always be one band corresponding to the probes used for amplification. A second band, equal in size of the sum of the sizes of the two probes. It will appear only when the target sequence, i.e., the concerned pathogen is present in the test sample.

LCR (i) is highly efficient; it detects as few as 200–300 target molecules in a sample. (ii) It is highly specific and rarely produces false positive signals, which is in contrast to PCR. (iii) The LCR procedure allows automated detection by employing fluorescence or hapten labeled probes. LCR has been used to detect a wide variety of infectious agents, e.g., *Chlamydia trachomatis*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeas*, herpes simplex virus (HSV), HIV, hepatitis B virus, Hepatitis C virus, etc.

During LCR, the blunt-ended probe duplexes, not paired to the target sequence, also become ligated at a low frequency; this is called target-independent ligation.

This limits the sensitivity of LCR to between 200 and 300 target molecules per sample. This problem can be overcome by using probe molecules having staggered ends; this is called gap ligase chain reaction (G-LCR). G-LCR is able to detect as few as 5 target molecules/sample. G-LCR also uses a thermostable DNA polymerase. Another modification of LCR, called asymmetric gap LCR (AG-LCR), also utilizes reverse transcriptase, and is specific for RNA detection.

Monoclonal antibodies

A monoclonal antibody (Mab) preparation is specific to a single antigenic determinant (epitope) of a single antigen. Mabs are usually produced from hybridoma clones; each such clone is derived by the fusion of a single myeloma cell with a single antibody producing lymphocyte. Mabs are being produced against a variety

of antigens and being employed for many purposes including diseases diagnosis. Mabs are employed for (i) classification of blood groups (ABO, Rh, etc.), (ii) clear and specific detection of pathogens, (iii) and a very early and accurate detection of cancers.

The immunological assays generally employed for diagnostic purposes are varied. Of these, ELISA is the most rapid convenient and highly efficient. More recently a technique called immuno-PCR has been developed; this can be used to detect rare antigens at the single cell level, and even single antigen molecules/sample can be detected.

Immuno-PCR technique uses PCR amplification of a marker DNA segment attached to an antibody for detection of the antigen for which this antibody is specific. The protocol of immuno-PCR may be stated in simple terms as follows:

- (i) Test samples suspected of containing the antigen are added to microtiter plate wells and the antigen is immobilized on the surface of wells.
- (ii) The free binding sites on the microtitre well surfaces are suitably blocked.
- (iii) The antibody specific to the antigen to be detected is added to the wells. It forms antigen-antibody complex; this occurs only in those wells where the antigen is present. Free antibodies are removed by washing.
- (iv) Now, the molecular linker is added; it binds to the fragment crystallizable region (Fc region) of the antibody. A streptavidin-protein A chimera is the most versatile molecular linker. This chimeric linker binds to the Fc domain of antibodies due to the protein A sequences. It also binds to biotinylated DNA molecules due to its streptavidin moiety. The linker molecule is already complexed with the marker DNA molecule when it is added to the microtitre wells.
- (v) A segment of the marker DNA is amplified by PCR.
- (vi) The PCR products are analyzed by gel electrophoresis. For a large-scale application, the PCR products can be labeled using fluorochromes and haptens which permit their rapid, even automated detection as in the case of LCR.

The antigen-antibody complex will be formed only in those microtitre wells which contain the target antigen (the antigen for which antibody employed in the test is specific). Therefore, PCR amplification will occur only in such wells; in other wells there will be no PCR products. Therefore, all test samples which yield PCR products will contain the target antigen.

It is critical that after each step, up to step (iv) a thorough washing is done to remove unbound and nonspecifically bound antibody and marker DNA molecules; this increases the precision of the assay.

Immuno-PCR is (i) highly precise and extremely sensitive; it is several orders of magnitude more sensitive than ELISA. Therefore, (ii) it can detect rare antigens and (iii) diagnose pathological conditions much earlier. It is (iv) extremely versatile so that it can be applied to even single cells, can yield quantitative estimates of the antigen, and is amenable to automation. In addition, (v) it is relatively simple.

Table 7.17 Few selected examples of autoantibodies used as disease-specific marker for diagnosis

Antibody	Antigen specific	Associated clinical condition
Anti-ds DNA	B form of DNA	Systemic lupus erythematosus
Anti-Jo- 1	Histidyl-tRNA synthetase	Polymyositis
Anti-RNA polymerase I	Subunits of RNA polymerase I complex	Scleroderma
Anti-Centromere	Centromeric proteins	CREST (a subset of scleroderma)
Anti-acetylcholine receptor	Acetylcholine receptor	Myasthenia gravis
Anti-mitochondrial	Pyruvate dehydrogenase complex	Primary biliary cirrhosis

^ads = double stranded

Autoantibodies are those antibodies that are specific to those antigens which are normally tolerated by the immune system, and are typical constituents of cells and tissues of the animal in question; such antigens are called autoantigens. Autoantibodies are produced in conditions of autoimmunity which may be simply described as ‘an attack by the immune system on the host itself’. These antibodies are either cell-specific or non-organ specific. They recognize a variety of cellular and subcellular components, including the components of replication, transcription, RNA processing, RNA translation, and protein processing. The antigenic specificities of autoantibodies can be useful in clinical diagnosis of the associated autoimmune diseases (Table 7.17).

Detection of genetic diseases

Human beings suffer from several hundred genetic diseases almost all of which are produced by single recessive mutations. Many of these ailments can be managed but there—is no cure for any of them, except for the fast emerging option of gene therapy (Sect. 7.5). Their incidence can be minimized by an early detection of the afflicted fetuses which are then aborted. Therefore, when a woman gets pregnant the probability of her having a child suffering from a genetic disorder is estimated based on the histories of her and her husband’s families, and from the knowledge of previous births, if any. In case of risk, further investigation is carried out for a clear-cut and specific diagnosis.

Obtaining fetal cells: Earlier fetal cells were obtained by amniocentesis, i.e., withdrawal of amniotic fluid (which has free cells of fetal or developing human embryo origin) with the help of a hypodermic syringe. But amniocentesis is applicable only 18 weeks or later after the pregnancy, which is rather late for an abortion. Therefore, fetal cells are now obtained from biopsies of trophoblastic villi which are an external part of the human embryo and later form a part of the placenta. The biopsy is performed during 6th or 8th week of pregnancy (using an endoscope passed through the cervix of uterus), usually provides 100 µg of pure fetal DNA.

Fetal cells present in the amniotic fluid obtained by amniocentesis are recovered by centrifugation and cultured to obtain sufficient cells for various analyses. But the

tissue obtained from a biopsy of trophoblastic villi is usually enough for assays and in Rico culture may not be necessary.

Disease detection: The fetal cells are used for detection of the genetic disorders in one of the following ways.

- (i) Determination of karyo type of cells provides information on various syndromes produced by gross chromosomal aberrations.
- (ii) Most of the genetic diseases produce defective proteins/enzymes or no enzymes; many of these proteins have been identified and some of these can be assayed. The fetal cells are used to assay the concerned enzyme activities to detect such genetic diseases. At least 35 genetic diseases can be detected by assaying activities of specific enzymes.
- (iii) In case of some genetic diseases, the concerned gene mutation may alter (either abolish or produce) the recognition site for a restriction enzyme. The RFLP so produced can be detected by Southern hybridization; a sequence of the concerned gene is used as a probe. For example, in case of sickle cell anemia, the mutation from GAG to GTG eliminates a recognition site for the restriction enzyme Mst II (CCTNAGG) in the β globin gene (β gene) of hemoglobin. DNAs from a normal (β^A) and the test individuals (including fetal samples) are digested with Mst II, subjected to gel electrophoresis and probed with a sequence of β globin gene. If the test individuals have normal β globin (β^A) gene, the bands detected in their Southern blots will be comparable to those of normal DNA. A sickle cell mutant of the β globin (β) gene will change this pattern in a detectable manner (Fig. 9.4). Heterozygotes will show the bands present in both normal and sickle cell DNAs.

This approach is applicable to only those disorders in which the gene mutation changes the restriction pattern. As a result, this approach is not of general application.

- (iv) A more general approach utilizes oligonucleotide probes representing the sequence altered by the gene mutation causing the genetic disease. Typically, a set of two separate probes are used for each disease: one probe is complementary to the normal sequence, while the other is complementary to the mutant sequence. The probes are radiolabelled and used to probe Southern blots; under appropriate conditions, the probes can distinguish the normal and mutant DNA samples.

A set of two 19-mer (19 base long) oligonucleotide probe has been successfully used to detect sickle cell anemia. One of the two probes (β^S probe) contains the sequence complementary to that changed by the sickle cell mutation, while the other (β^A probe) is complementary to the same segment of the normal allele. The Southern blots of normal individuals ($\beta^A \beta^A$) hybridize only with the β^A probe, those of sickle cell homozygotes ($\beta^S \beta^S$) only with the β^S probe, while those of the heterozygotes hybridize with both β^A and β^S probes. Similarly, other mutant genes (e.g., α -antitrypsin gene implicated in pulmonary emphysema) differing from the

normal allele for a single base could be detected using this approach. This approach is of more general application than detection of RFLPs. But, this assay can be used only in such cases where the base sequence of the gene segment containing the mutation (for both normal and mutant alleles) is known to allow the synthesis of the two oligonucleotide probes. Probes can be prepared for mutations due to base substitution, insertion or deletion in the concerned genes.

Disease treatment

Treatment of diseases utilizes a wide variety of preparations of both biological and abiological origins. The preparations of biological origin may either be crude (e.g., Ayurvedic medicines, some allopathic drugs, etc.) or purified to various degrees. Many of such compounds are obtained from plants, but a large number of them originate from microorganisms, cultured cells, and recombinant organisms.

Products from nonrecombinant organisms

Therapeutic agents from non-recombinant organisms may originate from the following systems.

Microorganisms. A large number of pharmaceuticals originate from microorganisms; they range from whole microorganisms, e.g., spores of *Lactobacillus* spore-genes, through biomass used as food/feed supplements, e.g., single cell proteins, to a variety of highly valuable compounds like antibiotics, vitamins, enzymes, organic acids, etc.

Plant cell cultures. Some biochemicals of pharmaceutical value are produced by cultured plant cells, e.g., shikonin, berberine, ginseng biomass, and Taxol. Taxol is produced from cell cultures of *Taxus* spp. grown in 75,000 l bioreactors and is used for the treatment of breast and ovarian cancers.

Animal cell cultures. Cultured animal cells are the source of several compounds used in the treatment of diseases, e.g., angiogenic factor, interleukin-2, β -interferon, etc.

Products from recombinant organisms

The products obtained from non-recombinant organisms are limited to their natural capabilities. Genetic engineering has, however, removed this limitation and genes from any organism can be transferred and expressed into any other organism. This has enabled the production of a large number of recombinant proteins, i.e., proteins produced by genetic engineering or recombinant DNA technology in microorganisms, cultured animal cells and, more recently, in plants (grown in the field).

Genetically engineered microorganisms. A large number of human genes encoding pharmaceutically valuable proteins have been cloned and expressed in microorganisms. Initially, *E. coli* was used as the host for obvious reasons of the ease in cloning. But yeast is fast becoming the host of choice for production of recombinant proteins. Several of the recombinant proteins, used for treatment of diabetes mellitus (protein, insulin), dwarfism [protein, human growth hormone (hGH)], cancer (proteins, interferons, interleukins, granulocyte macrophage colony-stimulating factor), thrombosis (streptokinase) and AIDS (e.g., interferons).

granulocyte macrophage colony-stimulating factor). Many other useful recombinant proteins are in advanced stages of development.

Animal cell cultures. More recently, cultured animal cells have been preferred for the expression of human genes encoding pharmaceutically valuable proteins. Some of these proteins have already been approved for therapeutic use, e.g., hGH (for dwarfism), tissue plasminogen activator (tPA; thrombolysis) erythropoietin (anemia), and blood clotting factor VIII (hemophilia); the last two of the proteins have been approved for marketing in India. In addition, more than a dozen recombinant proteins are in advanced stages of development.

Transgenic plants. Plants are highly desirable in many ways for commercial production of recombinant proteins of value. A large number of transgenes encoding pharmaceutically valuable proteins have been expressed in plants. There is at least one example of commercial production of a recombinant protein, hirudin, encoded by a synthetic gene expressed in *Brassica napus*. More examples are likely to follow in the near future.

Advantages. Production of recombinant proteins of pharmaceutical value in microorganisms, cultured animal cells or plants offers several important advantages over their conventional routes of production.

- (i) Production costs are reduced, in some cases drastically. For example, in case of interferon, the cost was reduced to about 150 times the cost of the conventional product derived from human blood.
- (ii) There is no risk of contamination by AIDS or any other virus as is the case with conventionally produced proteins, usually from human sources.
- (iii) They can be produced in far greater quantities than it is possible for conventional products.
- (iv) In some cases, proteins of human origin have become available, e.g., insulin, while conventionally only that animal origin could be obtained.

Interferons

Interferons (IFNs) are members of a large group of proteins called cytokines which affect a wide range of target cells and tissues by binding to specific receptors present on the surface of their target cells. In these and the following respect, cytokines resemble hormones: they are released into the bloodstream and other body fluids. But they differ from hormones in the following features: they are produced by a variety of cell types (and not by specific endocrine organs).

Interferons were originally identified due to their antiviral activity. The protection from viral infections by interferons is independent of the immune system. The interferons are of three major types: IFN- α IFN- β . IFN- γ and IFN- α group consists of a family of closely related proteins and is usually divided into two subfamilies. IFN- α I and IFN- α II, and is mainly produced by leucocytes. IFN- β is a single protein species, and is produced by both leucocytes and fibroblasts.

IFN- α and IFN- β share common receptors on the surface of target cells (Table 7.18), and are mainly induced by viral infections. In contrast, IFN- γ is

Table 7.18 Interferons, number of amino acids, and others

Interferon (IFN)	Number of amino acids	Produced by	Induced by	Receptors
IFN- α I subfamily	166–172	Leucocytes	Viral infection	IFN- α and IFN- β share common receptors
IFN- α II subfamily (= IFN- ω)	166–172	Leucocytes	Viral infection	
IFN- β	166	Fibroblasts and leucocytes	Viral infection	
IFN- γ	143	T-lymphocytes	Antigenic stimulation of T-lymphocytes	Distinct receptors

produced only by T-lymphocytes in response to antigen-activation, and has distinct receptors from those for IFN- α and IFN- β .

Mode of IFN action is not clearly understood, and more than one pathway may be involved. When IFN- α IFN- γ bind to their receptors one or more tyrosine kinases become activated. This leads to the phosphorylation and consequent activation of transcription factors like ISGF 3 (interferon-stimulated gene factor 3) which are then translocated from the cytoplasm into the nucleus. These factors then stimulate the transcription of a number of genes, including those encoding 2',5'-oligoadenylate synthetases, double-stranded RNA activated protein kinases (PKR) and Mx proteins. These and some other gene products protect the cells against a range of viruses. In addition, IFNs induce the following effects, the basis of which is not well understood.

1. Inhibition of viral replication by IFN- α IFN- β . IFN- γ .
2. Protection of cells against other intracellular parasites (all IFNs).
3. Inhibition of cell division in some normal and transformed cells (all IFNs).
4. Regulation of cell differentiation (all IFNs).
5. Induction of cytokines, e.g., IL-1, tumor necrosis factor, or colony-stimulating factors (by IFN- γ only).
6. Activation of macrophages by IFN- γ only.
7. Activation of natural killer cells (all IFNs) etc.

IFN- α is being used on a significant scale for the treatment of hepatitis B. In addition, interferons have been approved for use in the treatment of cancer and other viral diseases, including AIDS.

Growth factors

Growth factors belong to the group of proteins called cytokines—they can alter cell production, organogenesis, and disease susceptibility in animals. The effect produced by a growth factor will mainly depend on the presence of other growth factors, the target cells and their receptors on target cells. The nomenclature of

cytokines is confusing and terms like interleukins, growth factors, colony-stimulating factors, etc., are used often for a single protein. The term interleukin is the preferred one and new leucocyte products are designated by this name followed by a number, e.g., interleukin-13. The various growth factors can be grouped into the following families: (i) insulin like growth factors (IGF, e.g., IGF-I and IGF-II), (ii) nerve growth factors (NGF), (iii) epidermal growth factors (EGF), (iv) transforming growth factor β (TGF- β), (v) platelet-derived growth factors (PDGF), (vi) fibroblast growth factors (FGFs), (vii) hepatocyte growth factors (HGF), and (viii) hemopoietic growth factors (at least 16 cytokines; affect production and function of blood cells).

Many of the growth factors have been approved for treatment of human diseases. Erythropoietin (EPO) is used on a considerable scale for the treatment of anemia. Interleukin-2 in conjunction with LAK cells are being used for cancer therapy. Similarly, granulocyte macrophage colony-stimulating factor and granulocyte colony-stimulating factor are used to accelerate neutrophil recovery after chemotherapy or bone marrow transplantation. I2PO is also used to stimulate red blood cell production in kidney dialysis or cancer patients. Several other growth factors have been/are likely to be approved for similar and other applications.

Antisense nucleotides as therapeutic agents

A very effective and specific approach for the treatment of a variety of diseases is to design and use oligonucleotides (say 25–35 bases long) complementary to the 5' end of the parasite mRNAs; such oligonucleotides are called antisense oligonucleotides. The antisense oligonucleotide may be linked to an acridine for increased effectiveness. When such oligonucleotides were used on cultured blood parasite *Trypanosoma brucei*, the parasite was killed. This approach has been quite successful in the treatment of cancer, and antisense oligonucleotides are in various stages of evaluation.

Monoclonal antibodies

Monoclonal antibodies (Mabs) have several therapeutic applications, e.g., (i) to provide passive immunity against diseases, (ii) in treatment of diseases like leprosy (Mab preparations specific against the pathogen are administered at regular intervals), (iii) to deliver toxin molecules (as immunotoxins) specifically to cancer cells, and (iv) to deliver radioactivity to cancer cells.

Drug designing

This approach aims at designing drugs which specifically and selectively fit into the critical sites of the target molecules, thereby inactivating the latter. The target molecule may be an enzyme (concerned with either metabolism or DNA replication), a hormone receptor or some other important molecule involved in a disease. The aim of drug designing is to develop highly efficient drugs which have little or no side effects.

Drug delivery and targeting

Drugs are normally delivered either orally or parenterally (by injection). They become distributed in the whole body tissues and fluids, and only a small portion

reaches the diseased tissue/organ. This necessitates a much larger dose of expensive drugs, and may often produce severe undesirable effects in other organs/tissues. Further, oral route of drug administration is much more desirable than that by injection for obvious reasons. But this route is unsuitable for the new class of protein/peptide drugs due to poor uptake; this is because of proteolytic degradation in the gastrointestinal tract and poor permeability of the intestinal mucosa to these high molecular weight therapeutic agents. The following approaches are being developed for a more efficient and/or targeted delivery of drugs:

- (i) Peptide/protein drugs may be delivered by oilier routes, e.g., nasal, buccal, rectal, ocular, pulmonary, and vaginal routes; of these the nasal route appears to be the most promising. The epithelial membranes present barriers to drug uptake which can be overcome by using compounds that enhance permeability of these membranes. Some commonly used permeability enhancers are: sodium glycocholate, sodium deoxycholate, dimethyl- β cyclodextrin, etc. But prolonged use of these enhancers causes changes in the mucosal surface. Therefore, new and better enhancers are being developed.
- (ii) A variety of drugs can be encapsulated in liposomes, which are small lipid vesicles produced artificially. But liposomes have the disadvantages of larger size and poor tissue or cell type selectivity (liposomes become concentrated in liver and spleen). Tissue selectivity of liposomes can be greatly increased by attaching to their surface specific ligands, e.g., monoclonal antibodies (Mabs). When polyethylene glycol is also attached to the surface of such liposomes (having Mabs on their surface), the circulation time as well as the site-specific delivery of liposomes is greatly increased; such liposomes are called "stealth liposomes". Liposomes hold great promise as a DNA delivery system in gene therapy, but they have to be injected into the subjects.
- (iii) Polymers have been used as drug delivery systems; the drug is generally released by cleavage of the drug from the polymer, swelling of the polymer (for drugs trapped within the polymeric chains), through osmotic pressure generated pores, or simple diffusion. Polyesters are the widely studied biodegradable products; their hydrolysis yields nontoxic alcohols and organic acids. Polyesters of lactic acid and glycolic acid are the most widely used polyesters for slow release of drugs having large molecules, e.g., proteins, polysaccharides, and oligonucleotides. The drugs being delivered by the polymer systems include insulin growth factors, steroids, anticancer drugs, etc.
- (iv) The most effective system for site-directed delivery of drugs and other moieties (called drug targeting) is based on monoclonal antibodies. Immunotoxins serve as a good example of this approach. The application of this technology is limited by the low availability of Mabs specific to a given tumor cell type and also by the changing surface decorations (antigens displayed on the cell surface) of tumor cells. Intensive research efforts are directed in this very important area of disease treatment, and many important developments may be expected in the near future.

Artificial tissues/organs

Effective treatment of many ailments like burns, injuries, etc., requires tissue/organ transplants. Production of implantable tissues in vitro is called tissue engineering. Artificial skin produced in vitro is already in therapeutic use. It is hoped that artificial cartilage will also become available for therapeutic use in the near future. Studies are also focussing on the development in vitro of other organs like bone, liver, etc.

Gene therapy

Human beings suffer from more than 5000 different diseases caused by single gene mutations, e.g., cystic fibrosis, acatalasia, Huntington's chorea, Tay-Sachs disease, Lesch-Nyhan syndrome, sickle cell anemia, mitral stenosis, Hunter's syndrome, hemophilia, several forms of muscular dystrophy, etc. In addition, many common disorders like cancer, hypertension, atherosclerosis and mental illness seem to have genetic components. Malignant cells may arise due to mutations in two types of genes, viz., oncogenes and tumor suppressor genes; both the types of mutant alleles are involved in malignant transformation of cells.

Gene therapy may be defined in board general terms as follows: Introduction of a normal functional gene into cells which contain the defective allele of the concerned gene with the objective of correcting a genetic disorder or an acquired disorder. Application of gene therapy involves the following basic developments in genetics. molecular biology and biotechnology: (i) identification of the gene that plays the key role in the development of a genetic disorder. (ii) determination of the role of its product in health and disease, (iii) isolation and cloning of the gene, and (iv) development of an approach for gene therapy.

The candidate disorders for gene therapy are selected on the basis of the following criteria: (i) the disease should be life threatening, (ii) the gene responsible for the disease has been cloned, (iii) a precise regulation of the gene should not be required, and (iv) a suitable delivery system should be available.

Types of Gene Therapy

Gene therapy may be classified into two types: (i) germline gene therapy and (ii) somatic cell gene therapy. In case of germline gene therapy, germ cells, i.e., sperms or eggs (even zygotes), are modified by the introduction of functional genes which are ordinarily integrated into their genomes. Therefore, the change due to therapy is heritable and passed on to later generations. This approach, theoretically, is highly effective in counteracting the genetic disorders. However, this option is not considered, at least for the present, for application in human beings for a variety of technical and ethical reasons.

In somatic cell gene therapy, the gene is introduced only in somatic cells, especially of those tissues in which expression of the concerned gene is critical for health. Expression of the introduced gene relieves/eliminates symptoms of the disorder, but this effect is not heritable as it does not involve the germline. Somatic cell therapy is the only feasible option, and clinical trials have already started mostly for the treatment of cancer and blood disorders. This approach is divided

into two groups on the basis of the end result of the process: (i) addition or augmentation gene therapy and (ii) targetted gene transfer.

Augmentation therapy

In this type of somatic cell gene therapy, the functional gene is introduced in addition to the defective gene endogenous to the cell (s), i.e., the modified cells contain both the defective (endogenous) as well as the normal (introduced) copies of the gene. There are two general approaches to augmentation therapy. The first approach was used in the first two patients on whom gene therapy was attempted to correct the genetic disorder called Severe Combined Immune Deficiency (SCID) syndrome produced by adenosine deaminase (ADA) deficiency. (i) Normal/ADA gene copies were produced by cloning and then (ii) packed into a defective retrovirus; most of the viral genes were replaced by the ADA gene, (iii) Lymphocytes were isolated from the patients, and (iv) the recombinant retroviruses were used to infect the lymphocytes. Finally, (v) the infected cells expressing the ADA gene were injected back into the patients. The normal ADA gene was expressed in the patients, and ADA deficiency was partially corrected; this resulted in an improvement in the patient's immune system.

A variety of viral vectors have been used to deliver genes into target/stem cells. e.g., lymphocytes, bone marrow cells, cultured *in vitro*. The stem cells themselves are obtained either from the concerned patient (more desirable) or from a matched donor. The reservations about the safety of retroviral vectors are sought to be solved by developing suicide vectors which cannot replicate after the delivery of the gene. The other main problems of this approach are: (i) low frequency of transfection of stem cells, (ii) stability of the integrated gene, (iii) duration of the gene expression, (iv) lack of proper regulation of gene expression, etc. More recently, interest has focussed on physical methods of gene delivery like Ca^{2+} phosphate coprecipitation, particle gun, electroporation, etc.

The second approach is the direct injection of DNA into the tissues either as protein complexes (in order to bring about the receptor-mediated transfer of DNA into a specific tissue, e.g., liver) or even as naked DNA into muscle or skin. Interestingly, these cells take up the DNA and express the gene product. Exciting results have been obtained with experimental familial hypercholesterolemia, where LDL receptor levels have been augmented by injection of the gene as a sialoglycoprotein complex. The problems in this approach as well, relate to the frequency of cells taking up and expressing the gene and, more particularly, the duration of expression.

The gene delivery methods used for gene therapy can also be used for the treatment of cancer or AIDS. In case of cancer, a toxin encoding gene can be delivered into the cancer cells. Similarly, appropriate interleukin genes can be delivered to boost the body's defense mechanisms (in case of AIDS).

Targetted Gene Transfer

Targetted gene transfer or gene targeting uses homologous recombination to replace the endogenous gene with the functional introduced gene. The first case of such gene transfer was used to disrupt the human β -globin gene in cultured cells.

Subsequently, over 100 mammalian genes have been modified by this approach. Gene targeting can be used either to inactivate (by disruption) a functional endogenous gene or to correct a defective one. The initial gene targeting to disrupt the human β -globin gene used a double selection strategy called positive-negative selection.

The vectors employed for gene targeting are of two types: (i) insertion vectors and (ii) replacement vectors. The insertion vector is linearized by restriction cleavage within the sequence to be targeted; the targeted sequence provides the site for recombination and is different from the gene to be introduced. Hence, the sequence to be introduced is located in the inner region of the vector and is flanked by the sequences involved in recombination. A recombination of such a vector with its homologous cellular sequences produces a duplication of the targeted sequence; this is called insertional recombination. In contrast, a linearized replacement vector has the two halves of the target gene at its two ends. Recombination occurs within the two halves of the target gene, replacing a portion of the endogenous gene sequence by that of the introduced gene: this is called replacement recombination. There is no duplication of sequences, and the target gene becomes disrupted. Therefore, this approach can not be used for gene therapy.

A strategy has been devised to modify only a small sequence of the target gene without the attendant gene duplication/disruption produced by insertional/replacement recombination. This approach, called in-out method of gene targeting, consists of the following two steps:

- (i) The first step called "in" step, is targeted gene transfer using an insertion vector; the appropriately targeted cell will have a gene duplication.
- (ii) The second step, termed as "out" step, depends on either intrachromosomal recombination (between the introduced and the endogenous genes) or unequal sister chromatid exchange between homologous chromosomes. The recombination product of interest is a chromosome which has only a single and functional copy of the introduced gene.

The in-out strategy has been tested using the HGPRT (hypoxanthine-guanine phosphoribosyltransferase) gene. The gene was targeted into a mouse embryonic stem cell line; subsequently, it has been successfully used with some other genes. This procedure is ideal for gene therapy.

Gene targeting is the strategy of choice for gene therapy for the following reasons. (i) The targeted gene is changed in a precise and specific manner. (ii) The introduced functional gene is placed in the same context, i.e., it is flanked by the same DNA sequences, as the replaced endogenous gene. And, (iii) no other gene of the genome is affected. The major limitation of the approach is the low frequency of homologous recombination; this problem, however, is being removed by refinements of the technique. The feasibility of gene targeting has been demonstrated in a number of different cell types for several different genes. It is expected that targeted therapy would become feasibility for many genetic diseases in the near future.

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