1 Introduction

1.1. Overview of herbal drugs

Amongst the estimated 250,000-500,000 of plant species on Earth (Borris, 1996), only a small percentage (1-10%) is used as foods by both humans and animals, but even more are used for medicinal purposes (Moerman, 1996). Hippocrates mentioned about 300-400 medicinal plants in the late fifth century B.C. (Schultes, 1978). In the first century A.D., Dioscorides wrote *De Materia Medica*, a medicinal plant catalogue which became the prototype for modern pharmacopoeias. The Bible offers descriptions of approximately 30 healing plants. Indeed, frankincense and myrrh were reported to have antiseptic properties, and even employed as mouthwashes. In 1887, alternative practitioners compiled their own catalogues, notably *The Homeopathic Pharmacopoeia of the United States*. While the Asian cultures compiled their own pharmacopoeia, in the West the Renaissance years saw a revival of ancient medicine, which was built largely on plant products.

Due to varied chemistry of these natural chemicals they are widely valued all over the world. About 25% of the drugs prescribed worldwide originate from plants, and 121 active compounds are currently in use. In 1997, it was estimated that the world market over-the-counter phytomedicinal products was 10 billion US$, with an annual growth rate of 6.5%. The World Health Organization (WHO) considers phytotherapy in its health programmes and suggests basic procedures for the validation of drugs from

plant origin in developing countries (Vulto and Smet, 1988). Of the 252 drugs considered as basic and essential by the WHO, 11% are exclusively of plant origin and a significant number of synthetic drugs are obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* sp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna*, and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already in the market or under clinical trial are of natural origin. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be used as lead components, allowing design and rational planning of new semi-synthetic drugs.

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Countries, such as China and India, have a well-established herbal medicine industry, while Latin American countries have been investing in research programmes in medicinal plants and standardization and regulation of phytomedicinal products, following the example of European countries, such as France and Germany. Ayurveda, the traditional Indian medicinal system relies on using plant extracts, single and mixed preparations. The formulations often have lyrical names, such as ashwagandha (*Withania somnifera*) root, livo-vet etc. which are used to treat animals as well as humans (Dhuley, 1998).

Plants have a limitless ability to synthesize a wide range of secondary metabolites, such as terpenoids, steroids, alkaloids and predominantly phenols or their oxygen-substituted derivatives (Geissman, 1963). These compounds are widely spread in different parts of a plant, *viz.* fruits, seeds, leaves, flowers and barks. Plant phenolics mainly include derivatives and isomers of flavones, isoflavones, flavonoids, catechins, phenolic acids, quinines etc. However, the huge number of phenolics (~12,000), isolated from plants represents even less than 10% of their total natural abundance (Schultes, 1978). Besides phenolics, a diverse array of compounds, such as terpenoids, steroids and alkaloids also abounds in the plant kingdom. Some, such as terpenoids, give plants their odour; others (quinones and tannins) are responsible for the colour. Many compounds are responsible for plant flavour (*e.g.*, the terpenoid capsaicin from chili peppers), and several of the same herbs and spices used by humans to season food yield useful medicinal compounds (Table 1).

Amongst the diverse array of activities of the plant phenolics, major thrust is given to their antioxidant, antimicrobial and anticancer activities. The isoquinoline alkaloid, emetine, obtained from the underground part of *Cephaelis ipecacuanha* and related species, has been used for many years as amoebicidal drug as well as for the treatment of abscesses due to the spread of *Entamoeba histolytica* infections. Another important drug of plant origin with a long history of use is quinine. This alkaloid occurs naturally in

Table 1. Different classes of organic compounds and their medicinal properties

Class	Subclass	Example	Mechanism of action	Reference
phenolics	simple phenols	catechol, epicatechin	substrate deprivation and membrane disruption	Peres *et al.* (1997)
	phenolic acids, quinones	cinnamic acid, hypericin	binds to adhesion proteins and cell wall; inactivates enzymes	Duke (1985); King and Tempesta (1994)
	flavones, flavonoids	chrysin, abyssinone	binds to adhesion proteins and cell wall; inactivates enzymes; inhibits HIV reverse transcriptase	Brinkworth *et al.* (1992); Ono *et al.* (1989); Taniguchi and Kubo (1993)
	tannins	ellagitannis	binds to adhesion proteins and cell wall; inactivates enzymes; substrate deprivation; membrane disruption; metal ion chelation	Brownlee *et al.* (1990); Butler (1988); Haslam (1996); Scalbert (1991); Schultz (1988)
	coumarins	warfarin	interacts with eukaryotic DNA (antiviral activity)	Bose (1958); Hoult and Paya (1996); Keating and O'Kennedy (1997)
terpenoids, essential oil		capsaicin	disrupts cell membrane	Cichewicz and Thorpe (1996)
alkaloids		berberine, piperine	intercalates into cell wall and DNA	Burdick (1971); Houghton *et al.* (1994)
lectins, peptides		mannose-specific agglutinin, fabatin	blocks viral fusion or adsorption forming disulphide bridges	Meyer *et al.* (1997); Zhang and Lewis (1997)

the bark of *Cinchona* tree. Apart from its continued usefulness in the treatment of malaria, it can also be used to relieve nocturnal leg cramps. Currently, the widely prescribed drugs are analogues of quinine, such as chloroquine. Some strains of malarial parasites have become resistant to the quinines; therefore, antimalarial drugs with novel mode of action are required.

Similarly, higher plants have made important contributions in the areas beyond anti-infectives, such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblastine and vincristine, which were both obtained from the Madagascan periwinkle (*Catharanthus roseus*) (Nelson, 1982). Other cancer therapeutic agents include taxol, homoharringtonine and several derivatives of camptothein. For example, a well-known benzylisoquinoline alkaloid, papaverine, has been shown to have a potent inhibitory effect on the replication of several viruses including cytomegalovirus, measles virus and HIV (Turano *et al.*, 1989). Three new atropisomeric naphthylisoquinoline alkaloid dimers, michellamines A, B and C were isolated from a newly described tropical liana species, *Ancistrocladus korupensis* from the rainforest of Cameroon. These compounds showed potential anti-HIV activity, where michellamine B being the most potent and abundant member of the series. These compounds were capable of complete inhibition of the cytopathic effects of HIV-1 and HIV-2 on human lymphoblastoid target cell *in vitro* (Boyd *et al.*, 1994).

The first generation-plant drugs were usually simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state such as cinchona, opium, belladonna and aloe were selected as therapeutic agents based on empirical evidence of their clinical application by traditional societies from different parts of the world. Following the industrial revolution, a second generation of plant-based drugs emerged based on scientific processing of the plant extracts to isolate their active constituents. To isolate the active compounds, plant extracts are first qualitatively analysed by thin layer chromatography and screened for the biological activity. For purification and isolation, the active plant extracts are sequentially fractionated to yield pure compound(s). In general, a plant extract contains low

concentrations of active compounds, whose structures are determined by spectroscopic methods (NMR, IR, mass and UV). The second generation-phytopharmaceutical agents were pure molecules and some of the compounds were even more pharmacologically active than their synthetic counterparts. Notable examples were quinine from *Cinchona*, reserpine from *Rauvolfia*, and more recently taxol from *Taxus* spp. These compounds differed from the synthetic therapeutic agents only in their origin. They followed the same method of development and evaluation as other pharmaceutical agents.

In the development of third generation-phytotherapeutic agents, a top-bottom approach is usually adopted. This consists of first conducting a clinical evaluation of the treatment modalities and therapy as administered by traditional doctors or as used by the community as folk medicine. This evaluation is then followed by acute and chronic toxicity studies in animals. Studies should, when applicable, include cytotoxicity. It is only if the substance has an acceptable safety index; then it would be necessary to conduct detailed pharmacological/biochemical studies. Formulation and trial production of the dosage forms are structured to mimic the traditional use of the herbs. The stability of the finished product is given careful attention during the formulation of the final dosage form. This is a unique blend of the empiricism of the earlier first generation-botanicals with the experimental research used to prove the efficacy and safety of second generation-isolated pure compounds. Several pharmaceutical companies are engaged in the development of natural product drugs through the isolation of the so-called active molecules from plant extracts.

1.2. Aims of present investigations

Favourable climatic factors including the positive variations in humidity, temperature and altitude, and anthropogenic factors make the vegetation of Darjeeling Himalaya diverse and capable of sustaining a rich flora which serves as a storehouse of medicinal plants. The natives of this region are primarily surviving on folklore and cultural heritage. But the process of civilization is gradually eroding the knowledge of plants and their traditional usage in medicine. Many of the important information might have been lost or discarded *en route* to this age. The knowledge of folklore science, which remains limited within families of aborigines of such area, dies out with them. Recording of such knowledge of ethnobotany through surveys, recordings, specimen collection and examination along with interviews with the local tribal people and preservation of the acquired knowledge is the only means to restore the valuable ethnic information (Bhujel, 1996).

The health benefits of many of the plants/herbs may be related, at least in parts, to their antimicrobial and antioxidant properties. Also, these properties are often valued to increase the shelf-life of raw as well as semi-cooked food products. The plants used as ethnomedicines in Darjeeling Himalayan region are immense with great diversity. Thus, ascertaining the most suitable candidates with potential application as medicine and food preservative warrants extensive and proper screening. Hence, the objectives of the present investigation were to (1) survey various herbs used as ethnomedicines in Darjeeling hills, (2) evaluate their antioxidant activities using a series of *in vitro* assay and (3) screen the antimicrobial activities of the chosen herb extracts against a wide range of microorganisms (bacteria, yeasts and moulds).

The protocols adopted to fulfill these objectives were as follows:

(a) Surveying the three hilly subdivisions of Darjeeling district, documentation of the usage, local name etc. of the herbs and identification of their taxonomic status;

(b) Evaluation of antioxidant activity of a number of important herbs of this area using several *in vitro* assays;

(c) Screening of the above herbs for their inhibitory activity against a number of microbial strains; and

(d) Isolation of active fractions/compounds of some of the herbal extracts using various spectroscopic methods, and revalidation of their potency for the biological activity, mentioned above.