Chapter -1

1. INTRODUCTION

Herbal medicines which formed the basis of health care throughout the world since the earliest days of mankind are still attracting more and more attention within the context of health care provision and health sector reform. Recognition of their clinical, pharmaceutical and economic value is encouraging for international trading, though it varies widely between countries.

A number of plant based traditional system of medicines have been in use in India and also in many parts of the world for ages. Before the advent of modern medicine, the traditional systems of medicine were playing a central role in healthcare. According to an estimate, majority of the world population, especially in the developing countries, still depends on herbal products for their primary health care needs, possibly for the following reasons:

1. Lack of easy access to drugs of modern medicine.
2. Popular belief that herbal drugs are free of adverse effects
3. Most economic as against prohibitive cost of most of the allopathic drugs
4. Concern over the toxicity and side effects of modern drugs and
5. The holistic approach and belief towards the plant based medicines.

The traditional medicine has been steadily gaining interest and acceptance even amongst the practitioners of modern medicine also (Rajani and Padh, 2000).

Plants have also been a source of chemical substances which serve as drugs in their own right or as key ingredients in synthetic drugs. Many plant derived drugs used in modern medicine are developed by ethnomedical leads and subsequent ethnopharmacological studies. There are more than 100 drugs of known structure that are extracted from higher plants are included in modern medicine (Cox, 1994). One of the latest examples is the discovery

of taxol, a diterpene isolated from the stem bark of *Taxus brevifolia*, the most significant anticancer agent developed in the last two decades. The other examples include vincristine, vinblastine, etoposide, teniposide and artemisinin, etc.

A proper ethnopharmacological search and follow up studies can lead to many more useful drugs. Scientific studies available on a good number of plants indicate that promising phytochemicals (drugs) can be developed for many health problems. This phytochemical approach of plant discovery emphasizes the development of pure phytochemicals as drugs. The method is expensive and much time consuming (Gupta, 1994).

In phytotherapeutic approach, a fraction of an active extract or mixture of such fractions may prove better therapeutically, less toxic and inexpensive compared to pure isolated compounds. However crude plant preparations require modern standards of safety and efficacy.

1.1 Current status of traditional medicine

It was in the declaration of the International Conference on Primary Health Care held in Alma Ata, USSR, (WHO/UNICEF-1978) wherein the concept of primary health care was formulated with a positive acknowledgement of the role of traditional medical practitioners. A considerable interest in traditional medicine has been generated with the adoption of the resolution WHA-30.49 on "Promotion and Development of Training and Research in Traditional Medicine" and WHA-31.33 on "Drug policies and Management-Medicinal Plants" by the Thirtieth and Thirty First World Health Assemblies. Thus the importance of the traditional system of medicine and of certain traditional medical practices has now been recognized all over the world (Anonymous, 1978).

In the past decade there has been renewed attention and interest in the use of traditional medicine globally. In China, traditional medicine accounts for

around 40% of all health care delivered. In Chile 71% of the population, and in Colombia 40% of the population, have used such medicine. In India, 65% of the population in rural areas uses traditional medicines to help meet their primary health care needs. In developed countries, traditional, complementary and alternative medicines (referred to hereafter as "traditional medicine") are becoming more popular. For example, the percentage of the population that has used such medicines at least once is 48% in Australia, 31% in Belgium, 70% in Canada, 49% in France and 42% in the United States of America (WHO, 2002).

With the tremendous expansion in the use of traditional medicine worldwide people in the present time are more quality conscious and demand stringent quality control of consumer products. The aspect of assuring consistency and quality in herbal medicines has proved a bottle-neck in expanding business opportunities and delivering plant drugs from developing countries to the world market. The inherent problem of variation in the active content of plant drugs and the lack of easily available standards for medicinal plants have complicated the issue, which is on top priority of all governments in the developing countries (ICS-UNIDO, 2004).

The present trend demands increased emphasis on research on the quality of plant drugs and to unexplored plants of developing countries. These countries are rich in terms of traditional knowledge and natural resources. Irreparable loss from disappearing undocumented knowledge of healing with herbs and extinction of herbs call for steps to document traditional knowledge and scientifically explore their potential to discover useful drugs, which could provide value and valid reasons to stress conservation of plants which are medicinally important.

The increased demand of herbal products requires improved industrial activity in developing countries that are endowed with vast medicinal and aromatic plants (MAPs) resources. It promises them greater business

opportunities. At this juncture, there is the need to build strong interface to transfer scientific, technological and logistic support to developing countries in promoting their industrial activities on MAPs. The issue is more relevant to countries, which lack economic resources but have strong knowledge-base of medicinal herbs in health maintenance and rich in biodiversity (ICS-UNIDO, 2004).

1.2. Standardization of Herbal Medicines

India has a rich flora of about 16,000 species of flowering plants and it is estimated that about 7,500 herbs are used in local healthcare systems in rural and tribal areas of India. Out of these, the real medicinal value of over 4000 plants is either little known or hitherto unknown to the main stream population (Pushpangadan, 1995). In Indian systems of medicine there are about 1000 single drugs and 1500 formulations. These drugs are fully documented in traditional texts for their therapeutic properties. However, the difficulties in identification of appropriate plant species, their geographical source, time of collection, drying and storage conditions, microbial contamination and presence of xenobiotics, amongst other factors, that result in extensive and unpredictable variation in the quality of the raw material, which reflects eventually in the formulations as well.

With the commercialization of herbal medicines it has become necessary to undertake systematic studies on their quality, safety and efficacy levels. There are different ways in which countries define medicinal plants or herbs or products derived from them, and countries have adopted various approaches to licensing, dispensing, manufacturing and trading to ensure their safety, quality and efficacy (Xiaorui, 1998). Despite the use of herbal medicines over many centuries, only a relatively small number of plant species has been studied for possible medical applications. Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients and preparations containing them (Xiaorui, 1998).

Problems in standardization arise from the complex chemical composition of herbal drugs. Standardization in terms of certain marker compounds of an herbal drug in general does not serve the purpose of standardization, since activity of the drug does not depend upon one or a few chemical components. In most of cases, it is the result of concerted activity of several active compounds as well as of inert accompanying substances. Though these inert components do not directly affect the activity of the drug, they might influence bioavailability and excretion of the active components. Further, these inert chemical components may also play a role in the stability of the active component and minimize the rate of side effects. If there are several active compounds present in a herbal drug, they may have additive or potentiating effect (adjuvant effect). The quantity of the active constituents in the drug may be influenced by several factors such as age and origin, harvesting period and so on. To eliminate at least some of the causes of inconsistency, in terms of active ingredients, it was suggested that one should use cultivated plants rather than wild plants which are often heterogeneous with respect to the above factors and consequently in their content of active principles. Consistent quality of herbal medicinal products can only be assured if the starting materials are defined in an explicit and rigorous manner (Handa, 1995).

The quality of the herbal drug depends on the content of active constituents, the amount of which in turn depends up on various factors that affect the quality of crude drug. Wherever possible if the active constituent is known with certainty, it should be analyzed to assure the quality. However in many cases, information regarding active constituent is incomplete or active constituents are unknown. Under such circumstances, any one of the chemically characterized components of plant material called chemical marker can be used as a reference for evaluating the quality of the plant material. Thus the marker is a constituent of a medicinal plant material that is chemically defined and is of interest for quality control purpose, independent of whether they have any therapeutic activity or not. Most

appropriate biomarker is the active compound which is responsible for biological activity. When only inert chemical constituent are known from plant, a judicious selection of one of them for marker purpose should be made giving priority to a component specific to the plant under consideration and its stability.

Standardization involves "equalization of quality through all stages of processing starting with the herbal drug to the extract and the final product in order to achieve a defined standard". It is defined as adjusting the herbal drug preparation to a defined content of constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations (Harnischfeger, 2005).

At present, a wide variety of analytical techniques are available for quantification of various components of crude drugs. Chromatographic methods with wide range of sophistication are more important for phytochemical evaluation of the crude drugs. Of the many available chromatographic methods TLC has become widely accepted for rapid analysis of plant drugs. It can be used for qualitative as well as quantitative estimation of components of crude drugs. Qualitative determination can be carried out by fingerprint profiling of the extract. Quantitative estimation is carried out by TLC densitometry. TLC densitometry carries advantage over high performance liquid chromatography as it accepts comparatively unpurified samples without much compromise on the efficiency. More recently, a combination of chromatographic and spectroscopic method has become more popular for drug analysis.

World health organization in a number of resolutions emphasized the need to ensure the quality of herbs and herbal formulations by using modern techniques. Internationally and in our country too, several pharmacopoeias

have provided monographs defining quality parameters and standards for many herbs and herbal products.

A WHO consultation in Munich, Germany, June 1991, drafted Guidelines for the assessment of herbal medicines that were adopted for general use by the Sixth International Conference on Drug Regulatory Authorities (ICDRA) in Ottawa, October 1991. These guidelines (WHO/TRM/91.4) define basic criteria for evaluation of quality, safety and efficacy of herbal medicines. These also assist national regulatory authorities, scientific organizations, and manufacturers to undertake an assessment of the documentation, of submissions and/or the dossiers in respect of such products (WHO, 1991).

Several pharmacopoeias like, British Herbal Pharmacopoeia (BHP, 1983), The United States Pharmacopoeia (USP, 1990) include monographs for herbs to maintain their quality in their respective nations. Government of India too has brought out Ayurvedic Pharmacopoeia of India (1999) Vol I, part I, II and III which recommend basic quality parameters for common Ayurvedic drugs. The Indian drug manufacturer association also has come out with a compilation entitled 'Indian Herbal Pharmacopoeia' (IHP, 1998) composed of two volumes. Recently the European Scientific Co-operation for Phytotherapy (ESCOP) also has brought out a series of monographs on herbs. British Herbal Pharmacopoeia (BHP, 1983) brought out by British Herbal Medicinal Association in 1971 and later reprint of 1983, contained 233 monographs and quality controls tests for common herbs used in United Kingdom.

In most of these monographs along with identification tests like physico-chemical and microscopical characterization, there is also provision for gravimetric and titrimetric tests for a number of herbs. In some of pharmacopoeias, TLC fingerprint for a number of herbs is recommended (Dobriyal and Narayana, 1998) but they lack in tests based on modern

analytical techniques like chromatography, spectroscopy and analysis of marker content.

Phytochemical standardization using sophisticated analytical techniques is a must to ensure quality and efficacy of herbal drugs. Phytochemical standardization can be done at two levels:

1. Standardization by analysis of chemical/biomarker compound
2. Standardization using fingerprint techniques/ chemoprofiling

These are described in the following sections.

Every herb has a range of chemical constituents which are produced as a result of metabolic activities in the plants. These compounds either alone or in combination may be mainly responsible for the pharmacological activities or therapeutic effects. Hence it would be more practical to test for the presence of these compounds. Aswagandha (*Withania somnifera*) is assayed for withanolides, Guggulu (*Commmiphora mukul*) for guggulusterones, Neem (*Azadirachta indica*) for azadirachtin or nimbidine, Haridra (*Curcuma longa*) for curcuminoids, etc. are some of the herbs which are standardized interms of active principles (Dobriyal and Narayana, 1998).

On the other hand, where the chemical composition of the herb is worked out but it is not clearly established whether these chemical entities are responsible for some particular action, any compound which is predominantly present in that herb can be utilized as marker compound for the purpose of standardization. Aegelin in bilva (*Agle marmelos*), shatawarine in shatawari (*Asparagus racemosus*), fistulin in Aravadha (*cassia fistula*), etc are some of the herbs standardized using content of these markers (Dobriyal and Narayana, 1998).

Chromatography is the one of the most reliable methods for standardization of herbs and herbal formulations. It helps not only in establishing the correct identity but also in maintaining the chemical sanctity of the herbs. Gas Chromatography and High Performance Liquid Chromatography are generally preferred for quantification of volatile and non volatile compounds

respectively. In the past few years, HPTLC has also emerged as a potential tool for rapid and efficient analysis of extracts of herbal drugs and formulations (IHP, 1998; Shah et al., 2000).

The process of chemo profiling or fingerprint technique essentially involves steps like (1) collection of plant material from different geographical locations (2) preparing different extracts (by successive solvent extraction of the plant material with different solvents) and (3) establishing the chemical pattern of components present in the extract using chromatographic techniques like HPLC and HPTLC. The pattern thus obtained from the above process is unique for a particular plant (Bhutani, 2000).

1.3. Plants as adjuvants

Many medicinal plants classified as 'Rasayana' in Ayurveda are believed to be useful in strengthening the immune system of an individual (Patwardhan et al., 1990). Ayurveda (with particular reference to plants) may play an important role in modern health care, particularly where satisfactory treatment is not available with the use of single drug entities. Many herbs are also used in Ayurveda as adjuvants to counteract side effects of modern therapy and compare the cost effectiveness of certain therapies vis-à-vis modern therapeutic schedules.

It was revealed that the co-administration of certain herbs in chemotherapy reduces the toxic effects of chemotherapeutic agents and the therapeutic effects can be enhanced. For example, silymarin compounds have reduced the typical toxic effects of cisplatin on kidneys in animals without compromising the drug's anti-tumor activity. In case of adriamycin these have also been found to increase the drug performance (Gaedeke et al., 1996; Scambia et al., 1996).

The available information on the medicinal plants that are used in many herbal formulations sold in the market for variety of disorders showed that

some of these do not fall under any specific category of afflictions but act as supportive measures in treatment of diseases.

The Ayurvedic texts in our country categorize some plants as adjuvants, which generally act as supplement in a preparation like *Chvayanprash Avaleha*, herbal formulations like Geriforte, LIV-52 etc. The plants which fall under this category are believed to act as tonic, adaptogenic, immunomodulator's, healing agents in injuries and also bioactivity enhancers. Many drugs, under the category of rasayanas of Ayurveda, have been used for this purpose like ashwagandha, shatavari, guduchi, pepper, amla, neem, chitrak etc. The dried seeds (Semen Cuscutae) of *Cuscuta chinensis* Lam. (Convolvulaceae), an important traditional Chinese herbal medicine are widely used as a tonic and aphrodisiac, to nourish the liver and kidneys and to treat impotence and seminal emission (Xiong and Zhou, 1994; Du et al., 1998). Ethanol extract of Semen Cuscutae was found to possess potential adjuvant effects on the cellular and humoral immune responses against ovalbumin (OVA) immunization in ICR mice (Hang-Jun Pan et al., 2005). In a similar study it was observed that saponins of *Panax notoginseng* possess immunologic adjuvant activities with low haemolytic effect by enhancing the humoral and cellular immune responses to OVA in mice when given together with OVA (Xiang et al., 2003).

Many plants used in Indian system of medicine are also reported to be used in the treatment of impotence, seminal debility, as aphrodisiac, as fertility regulating agents and in other reproductive disorders. Important Ayurvedic texts available today (such as Charak, Shishruta, Bhavaprakash Nighantu etc) have given due importance to aphrodisiacs (Gian and Tapan, 1998). Out of various preparations described as aphrodisiac by Bhavaprakash fourteen are of plant origin (Misra, 1969). 'Indian Materia Medica' provides a long list of products used as aphrodisiac (Joshi and Nagaraju, 1991; Nadkarni, 1976). As many as seventy seven plants mentioned as aphrodisiac in the

indigenous system of medicine are also described in literature (Puri and Quart, 1971).

Some of the plants that are used as aphrodisiac are *Asparagus recemosus* (Bhatnagar et al., 1973; Sudhakar and Rao, 1985), *Moringa olifera* (Bhatnagar et al., 1973), *Mucuna pruriens* (Thakur et al., 1992), *Sida cordifolia* (Bhatnagar et al., 1973), *Tribulus terrestris* (Varma, 1982; Gauthaman et al., 2002), *Trichopus zeylanicus* (Subramoniam et al., 1997) etc.

Medicinal plants used to regulate male fertility are the other class of traditional drugs in the Indian system of medicine that act on male reproductive system and the need for research on male fertility regulation is recognized world wide. World Health Organization (WHO) Special Programme for Research Development and Training in Human Reproduction has a task force for the method of regulation of male fertility (Griffin, 1989). The task force is aiming to provide this choice through a research programme involving different research strategies. The importance is given mainly for (1) suppression of sperm production by hormonal means, including the development of long acting androgen supplementation, (2) search for drugs with an action on spermatogenesis or on sperm maturation in the epididymus, (3) collaborative research on new leads of plant origin. In addition to its own research strategy, the task force is active in encouraging the interest, raising the awareness and increasing the competence of scientists to do good research (Shah et al., 1997). In this regard many sex steroids were made available for suppression of spermatogenesis as a reversible chemical method of male contraception. The following hormones have been used: danzol (Dixit et al., 1981), depot medroxy progesterone acetate (DMPA) (Bhiwagade et al., 1991; Rao and Ray, 1993), cyproterone acetate (Wu, 1997), levonorgstral (Bebb et al., 1996), Ig-nortestosterone (Nagata et al., 1999), testosterone buciclate (Behre et al., 1995) and other androgen esters (WHO, 1996; Wu et al., 1996).

An ideal male contraceptive should be safe, effective and reversible. Above steroids were found to be effective in suppression of spermatogenesis but their treatment caused unacceptable side effects with long term use. Therefore much importance is given towards the search of male contraceptives from traditional drugs. In this regard number medicinal plants were screened for antifertility properties, mainly, for their effectiveness on reduction of sperm count, sperm motility and the overall suppression of spermatogenesis. The following plants are reported as effective antifertility agents: *Embelia ribes* (Purandare et al., 1979; Agarwal et al., 1986), Azadirachta indica (Shaik et al., 1993; Kasturi et al., 1995), *Abrus precatorius* (Kulshreshtha and Mathur, 1990), *Carica papaya* (Udoh and Kehinde, 1999; Lohiya et al., 2002), *Trypterygium wilfordii* (Qian, 1987; Lue et al., 1998). A number of review articles are also published by many authors on the list of plants that were used by different tribes and folk systems of medicine as male contraceptives or help regulating the male fertility (Shah et al., 1997; Kamal et al., 2003; Jain et al., 2004).

In India Ayurvedic and other plant-derived drug products that are used either as active ingredients or as adjuvants hold paramount importance as alternative medicines but, their standardization poses a great challenge to the practitioners as well as the consumers. This problem multiplies many more folds especially when these plants are known by similar vernacular names, leading to controversy in their identification.

A number of Indian medicinal plants used extensively in Ayurvedic or herbal medicines fall under the category of controversial sources. This is due to lack of their identity parameters or some other reasons as mentioned below. An example is *Rasna* (a species hailed for its effectiveness in the treatment of rheumatism) for which at least three different plant species are supplied as source, viz., *Vanda roxburghi, Pluchea lanceolata* and *Saccolabium papillosum* (Anonymous, 2000; Chopra et al., 1994).

The controversy of source of the herb may be due to

1. Confusion in vernacular names between indigenous systems of medicine and local dialects.
2. Lack of knowledge about the identity of the plant.
3. Non-availability of the authentic plant.
4. Similarity in morphology and aroma, etc.

In Ayurveda, 'Parpatta' refers to *Fumaria parviflora*. In Siddha 'Parpadagam' refers to *Mollugo pentaphylla* (Anonymous, 2000; Chopra et al., 1994). Owing to the similarity in the names in traditional systems of medicine, these two herbs are often interchanged or adulterated or substituted. Because of the popularity of Siddha medicine in some parts of south India, traders in these regions supply *M. pentaphylla* as 'Parpatta/Parpadagam' and the north Indian suppliers supply *F. parviflora*. These two can be easily identified by the presence of pale yellow to mild brown colored, thin wiry stems and small simple leaves of *M. pentaphylla* and black to dark brown colored, digitate leaves with narrow segments of *F. parviflora*. *Casuarina equisetifolia* for *Tamarix indica* and *Aerva lanata* for *Bergenia ciliata* are some other examples for controversial identity due to confusion in common names.

Nagakesar' is one of the important drugs of Ayurveda. The authentic source is *Mesua ferrea* (Anonymous, 2000). However, market samples are adulterated with flowers of *Calophyllum inophyllum*. Though the authentic plant is available in plenty throughout the Western Ghats and parts of Himalayas, suppliers are unaware of it. There may also be some restrictions in forest collection, and due to these reasons, *C. inophyllum* (which is in the plains) is sold as 'Nagakesar'. Authentic flowers can be easily identified by the presence of two-celled ovary whereas in case of spurious flowers they are single celled.

Hypericum perforatum is cultivated and sold in European markets. In India, availability of this species is very limited. However, the abundant Indo-Nepal species *H. patulum*, sold in the name of *H. perforatum*. Market sample is a whole plant with flowers and it is easy to identify them taxonomically. Anatomically, stem transverse section of *H. perforatum* has compressed thin phloem, hollow pith and absence of calcium oxalate crystals. Whereas *H. patulum* has broader phloem, partially hallow pith and presence of calcium oxalate crystals.

'Vidari' is another example of controversial category of drugs. *Pueraria tuberosa* and *Ipomoea digitate* are described as its sources (Anonymous, 2000). Interestingly, market samples are not derived from any of these two while an endangered gymnosperm *Cycas circinalis* is sold in plenty as 'Vidari'.

Some of the other most common controversial drugs are like, *Brahmi* (*Bacopa monnieri*, family: Scrophulariaceae; *Centella asiatica*, family: Umbelliferae) *Ashwagandha* (*Withania somnifera* and *W. ashujagandha* of the family Solanaceae), *Shankapushpi* (*Convolvulous pluricaulis* and *Evolvulous alsinoids* of the family Convovulaceae), *Jeevanti* (*Leptadenia reticulata*, family: Asclepidiaceae; *Holostemma adakodien* of the family Asclepidiaceae, *Flickengeria macraei*, family: Orchidaceae); Vidarikand (*Pueraria tuberose*, family: Fabaceae; *Ipomea digitata*, family: Convolvulaceae), etc. Some of these belong to same genus but of different species or some have different genus or sometimes belong to different families.

The present studies pertain to one such similar drug known as *"Brahmadandi"*, a controversial drug of Indian system of medicine. It has been used from long time and acclaimed beneficial in various diseases. According to the available literature on this drug the name Brahmadandi is associated with different plants (Vaidya, 1982; Issar, 1974; Nadkarni, 1976).

The plant material commonly available and sold in different markets of India as brahmadandi consists of the roots of *Echinops echinatus* Roxb (Compositae) and also in some places aerial parts of *Tricholepis glaberrima* DC (Compositae). The drug is popular as aphrodisiac in the treatment of male infertility, as is supported by a report on the aphrodisiac properties of the plants belonging to the family Compositae (Gian and Tapan, 1998). It is used as a single plant remedy or in polyherbal formulations (Nadkarni, 1976), particularly in organized systems of medicine such as Ayurveda, Unani, Homeopathy (Kirtikar and Basu, 1975), and other systems of medicine including the folk, ethno or tribal medicines. **Though the drug 'Brahmadandi' is popular for its medicinal uses, scientific data to ensure its identity, quality, safety and efficacy are still unavailable.**

Steps have already been taken up towards establishing the standards for many Indian medicinal plants but a number of plants have left untouched although these are used widely in the traditional systems of medicine. **Therefore it was proposed to undertake, first, the systematic investigations on the selected plants in order to evolve certain parameters for their standardization and secondly, to assess their biological activities as a means of biological standardization and thereby to justify their traditional claims.**

1.4. Profile of the selected plants

In order to develop the methods for standardization of some commonly used medicinal plants, the leads were selected by consultation with the traditional medical practitioners and from Ayurvedic literature. Roots of ***Echinops echinatus*** and aerial parts of ***Tricholepis glaberrima*** were taken for the study.

1.4.1. *Echinops echinatus* Roxb.

Family: Asteraceae (Compositae)

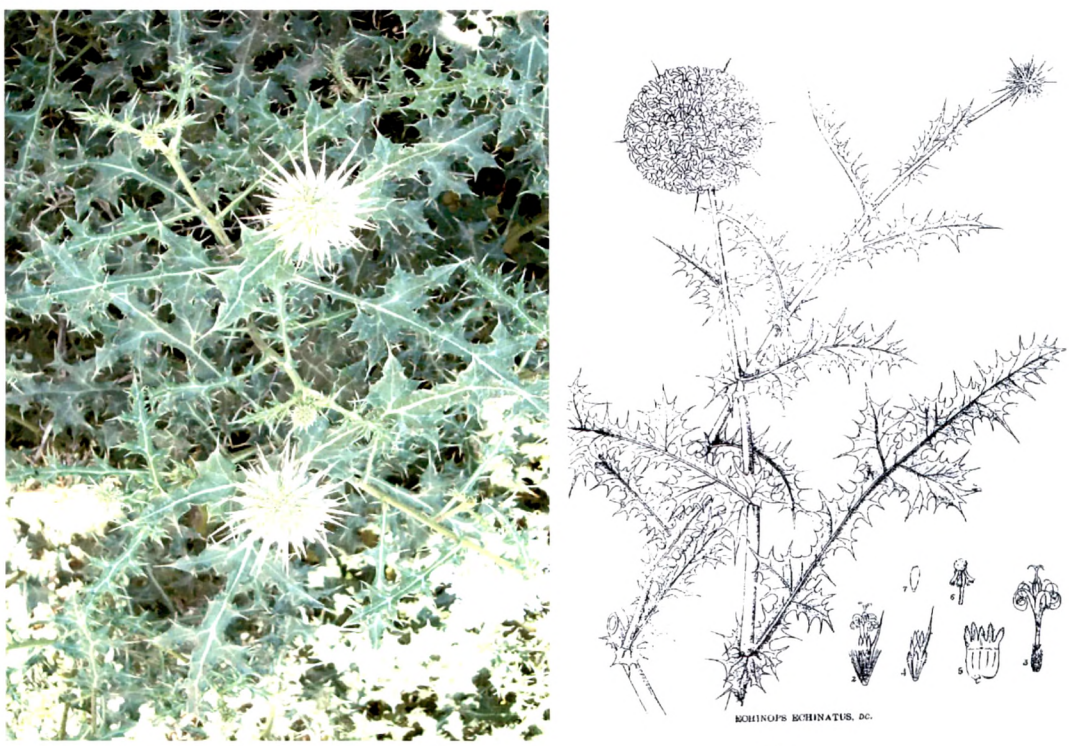
Common (Indian) names

Sanskrit	: Utakantaka; Brahmadandi; Ajadandi; kantalu
Hindi	: Untakatara
Gujarati	: Utakanto; Shuliyo
Kannada	: Brahmadande
Marathi	: Kadechubak; Utanti
English	: Camel's thistle

Habitat and distribution: The species is found practically throughout India Pakistan and Afghanistan. It is a xerophytic annual herb found on the hills (Kirtikar and Basu, 1975; Khan et al., 2000).

Description

The plant is a much branched rigid annual herb of 0.3-0.9 m height; branches widely spreading from the base with cottony pubescence (Figure 1.1). Leaves are sessile, 7.5-12.5 cm long, glabrous or minutely scaberulous above, white with cottony wool beneath, oblong, deeply pinnatified, the lobes are triangular and oblong, sinuate and spinescent, the spines often 2.5 cm long. Balls of the heads white, normally 2.5-3.8 cm in diameter (excluding the spines (Figure 1.1). Involucres surrounded by strong white bristles resembling pappus hairs; outer involucral bracts oblanceolates;



a b

Figure 1.1. *Echinops echinatus* Roxb.

intermediate bracts with 1 or 2 of the bracts often produced into sharp spines sometimes exceeding 2.5 cm long, causing the balls frequently to bristle with many spines. Pappus short and yellowish; forming a short cylindric brush above the achene. Achenes 4 mm long, obconic, densely villous (Kirtikar and Basu, 1975; Anonymous, 1952).

Parts used: Roots and root bark; Leaves; whole plant.

Traditional uses: The plant is pungent, bitter, and hot; stomachic, antipyretic, analgesic; improves the taste; increases the appetite; stimulates the liver; useful in diseases of the brain; diseases of the heart; pains in the joints; inflammations. The root is abortifacient and aphrodisiac (Kirtikar and Basu, 1975); used in the hoarse coughs of children; infusion is given in seminal debility, impotence, hysteria, etc (Nadkarni, 1976).

Folklore uses: Traditional healers of Chhattisgarh in India use this herb in different ways both internally and externally for the treatment of sexual disorders. An aqueous paste of the root is applied in the lower abdominal region to hasten the process of delivery; also the patients are advised to take the paste internally for quick and safe delivery. In case of patients having poor sexual vitality, aqueous paste of the root bark powder is applied externally on the male genitals one hour before intercourse; pure honey can be used in place of water for better results. A paste prepared by mixing the root bark powder with the juice of *Datura stramonium* and *Blumea lacera* leaves is used to avoid premature ejaculation. The patients suffering from respiratory troubles, particularly asthma, are advised to inhale the fumes obtained by burning the leaves and roots of *Echinops echinatus* Roxb in order to get quick and permanent relief (Oudhia, 2003a). In Karachi and Sindh region of Pakistan the plant has been reported to be used as a male contraceptive (Shah et al., 1997). Infusion of the plant is given orally to speed up the child birth (Kakrani and Saluja, 1993).

According to an ethnomedicinal survey carried out by Kakrani et al (2005), the rural population of Kutch region in Gujarat state, India, uses the suspension of root bark powder in milk (100 g/ 250 ml) for the treatment of diabetes. The bark of the roots of *E. echinatus* and the seeds of *Chohara* (Dates) in equal amount are crushed into powder and a tablespoonful every day is used to cure stomach diseases. Roots of *Untkatara* (15 g) are wrapped in a clean cloth and dipped in a container having half litre milk and one litre of water to this 25 g of *Chohara* (Dates) was added and boiled. When only milk remains 1–2 table spoonful of sugar is added to the milk, this milk is used as aphrodisiac.

In an indigenous formula the powdered root is made into paste and is applied on the belly of a pregnant woman at the time of child birth; it is considered helpful for easy delivery of the baby (Khan et al., 2000). In a survey of tribal area of southern Rajasthan carried out during the period of 2001-2002 similar use was reported viz., roots are put in the hairs of the head for easy delivery and are removed immediately after delivery otherwise uterus may come out (Jain et al., 2004). Another survey reports a paste prepared from the roots was applied on the head for the same purpose (Singh and Ali, 1992; Sikarwar and Kaushik, 1993).

A mixture (1:2) of *Tribullus terrestris* fruit and *Curculigo orchioid* underground part is given with *E. echinatus* root juice to treat acute debility after child birth (Shah and Gopal, 1985). A decoction prepared by boiling the root bark of *E. echinatus* with stem bark of *Tinospora cordifolia*, stem bark of *Wrightia tinctoria* (40 g) and seeds (15-20 g) of *Nigella hispanica* was used to treat fever (Reddy et al., 1989). Powder of the herb was administered three times a day for three days to cure colic (Singh and Maheshwari, 1994).

Pharmacological activities: Methanol, benzene and hexane extracts of *E. echinatus* roots were reported to exhibit antibacterial and antifungal activities against a number of organisms (Savitasharma and Mehta, 1989).

Hydro alcoholic extract (50%) of the aerial parts of *E. echinatus* was reported to possess hypoglycemic and diuretic activities when administered orally at the dose of 250 and 750 mg/kg body weight respectively in rats (Abraham et al., 1986). Whole plant extract of *E. echinatus* and an isolated compound from this plant viz., taraxasterol acetate were reported to possess anti inflammatory activity in several arthritic models in albino rats (Singh et al., 1989; Singh et al., 1991).

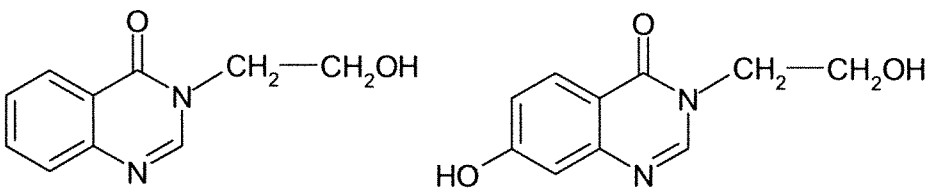
Experimental studies in female rats showed that the antifertility effects of *E. echinatus* roots and its various extracts are due to antiestrogenic activity (Savitasharma et al., 1988) whereas alcohol extract of *E. echinatus* roots exhibited antifertility effects in male rats when administered orally at the dose of 200 mg/kg body weight (Chaturvedi et al., 1995; Kamal et al., 2003). Four phenolic compounds, viz., apigenin, apigenin-7-O-glucoside, echinacin, echinaticin, were isolated from the whole plant of *Echinops echinatus* Roxb and were screened for antifungal properties against *Alternaria tenuissima*. All showed high efficacy against the pathogen at concentrations ranging from 25 to 150 µg/ml. Echinacin, which was highly effective at a concentration of 150 µg/ml was considered to be most promising of all compounds (Singh et al., 1988).

Phytochemical studies:

Two *O*-glycosyl flavones were isolated from the alcohol extract of the aerial parts of *E. echinatus*. Apigenin 7-*O*-β-D-glucoside was obtained by fractionation of alcohol extract with chloroform where as the second compound viz., Apigenin 7-*O*-β-D-(4''-cis-*p*-coumaroyl) glucopyranoside was obtained by extraction of alcohol extract with n-butanol (Chaudhary and Thakur, 1986).

Chemical investigations on the aerial parts of the *E. echinatus* led to the isolation of two known alkaloid viz., echinopsine and echinopsidine along

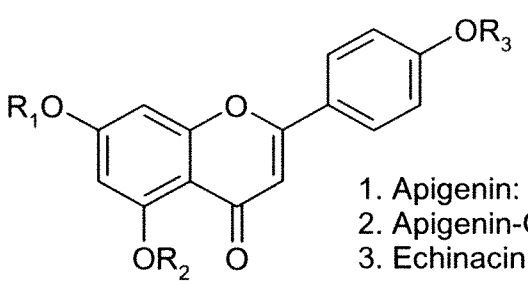
with a new alkaloid viz., echinozolinone having 4-quinozolinone skeleton (Figure 1.2).



Echinozolinone 7-Hydroxy echinozolinone

Figure 1.2

This was the first report on the occurrence of 4-quinozolinone alkaloid in Compositae (Chaudhary, 1987). The flavones Apigenin, Apigenin-7-*O*-glucoside, echinacin (Figure 1.3) and echinaticin were isolated from the alcohol extract of the whole plant of *E.echinatus* by repeated column chromatography and crystallization (Singh et al., 1988).



1. Apigenin: R1= R2= R3= H
2. Apigenin-O-glucoside: R1 = Glucose, R2 = R3 = H
3. Echinacin: R1 = H1, R2 = R3 = Me

Figure 1.3

A new isoflavone glycoside, echinoside, together with 7-hydroxyisoflavone, kaempferol-4-*O*-methylether, kaempferol-7-methylether, myrecetin-3-*O*-α-L-rhamnoside, kaempferol and kaempferol-3-*O*-α-L-rhamnoside, has been isolated from the whole plant of *Echinops echinatus* (Singh et al., 2006).

A triterpenoid viz., taraxasterol acetate was isolated from the alcohol extract of the whole plant of *E. echinatus* (Singh et al., 1991).

Studies on the flowers of *E. echinatus* showed that the basic fraction of the methanol extract of the flowers upon chromatographic separation led to the characterization of a minor alkaloid viz., 7-hydroxy-3-(2-hydroxyethyl)-(3*H*) – quinozolin-4-one as its hydrochloride (Figure 1.2) (Chaudhary, 1992).

The plant was also reported to contain beta amyrin, hentriacontane and hentriacontan-1-ol (Sen et al., 1968), β –sitosterol and betulinic acid (Hogale and Uthale, 1988), daucosterol (Singh and Pandey, 1989). The flowers are reported to contain echitin (Ram et al., 1996), luteolin, palmitic acid, quercetin, lupeol and lupeol acetate (Chaudhary., 1988).

The only report available on the chemical constituent of the roots showed the presence of allophanic acid (Sharma et al., 1988).

1.4.2. *Tricholepis glaberrima* DC.

Family: Asteraceae (Compositae)

Common (Indian) names

Sanskrit	: Ajadandi; Brahmadandi; Kantapatraphala
Hindi	: Brahmadandi
Gujarati	: Brahmadandi; Phusiarun; Talakanto
Marathi	: Brahmadandi; Motachor
Bengal	: Chhagaladandi; Vamanadandi
Bombay	: Motabor

Habitat and distribution: It is an annual herb, found in Western Rajputana, Mt. Abu, Central India, Konkan, Deccan, and Western Ghats in

the Bombay presidency, South Canara, Coorg and in the hills of Mysore (Anonymous, 1976; Chopra et al., 1956).

Description

The plant is quite glabrous; stem erect, slender and as well as the branches angled and ribbed (Figure 1.4). Leaves sessile 2.5 - 6.3 cm long and 0.5 - 1.5 cm wide, linear - oblong or lanceolate, acute, entire, spinous toothed or spinous - serriate, punctuate, base of the cauline leaves not or rarely auricled; midrib and nerves very prominent beneath. Heads 1.5 – 2 cm, long, ovoid, glabrous, corollas 1.25 - 1.4 cm long, purple, involucral bracts linear - lanceolate, aristate-acuminate, ciliolate, sub-erect or slightly recurved. Style arms slender, with a ring of hairs at the base of the lobes. Palea of the receptacles reaching much above the pappus, narrowly linear or acute. Pappus shorter than the achenes, copious. Yellowish brown, rigid, subpaleaceous. Achenes oblong, faintly ribbed (Kirtikar and Basu., 1975).

Parts used: Whole plant, Roots and root bark.

Traditional uses: The plant is reported to be hot and bitter; cures *Kapha*, *Vata*, inflammations; used in leucoderma and in skin diseases. It is believed to be nervine tonic, aphrodisiac and is used in seminal debility (Kirtikar and Basu., 1975). The herb is believed to be antiseptic whereas the root bark is reported to be used in cough and in treating the troubles of urinary tract (Anonymous, 1976). Root paste is reported to be used in snake bite (Bedi, 1978).

Folklore uses:

Aerial parts of *T. glaberrima* are used as crude drug in various indigenous systems of medicine for treating different ailments. According to a report on an ethnomedicinal survey, the traditional healers of Chhattisgarh in India use this herb in different ways both internally and externally for the treatment of sexual disorders.

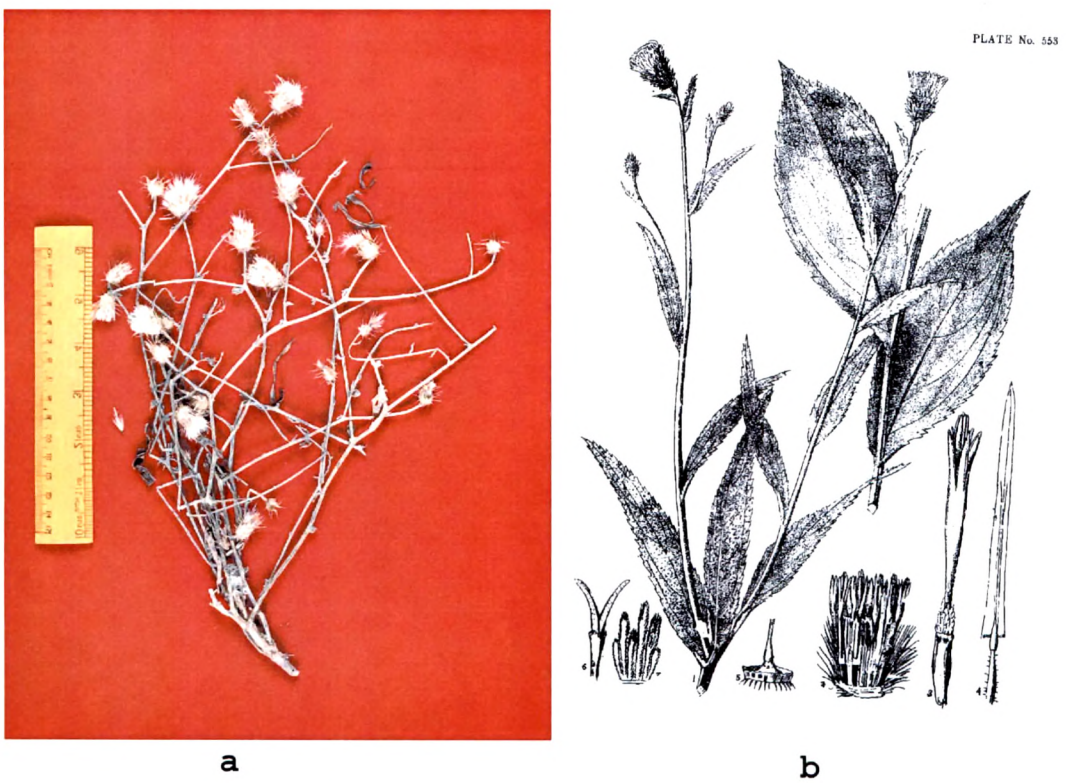


Figure 1.4. *Tricholepis glaberrima* DC.

Traditional healers collect the whole herb from fields and dry it in shade, after complete drying the whole herb is converted into powder. The whole herb powder is recommended with *Kali mirch* (Black pepper) in the treatment of diabetes and also used as sex tonic to be taken twice a day with a glass of cow's milk (teaspoonful) for quick results. It is considered as 'Poor man's sex tonic' by many healers who also use the root powder with *Asgandh* (*Withania somnifera*) root powder and *Gud* (Jaggery) to get powerful effect. The fresh juice of *Brahmadandi* is a boon for women having the problem of *Safed paani* (leucorrhoea), fresh juice of the whole herb or its powder is given with cow milk till complete cure (Oudhia, 2003b).

Phytochemical studies:

Petroleum ether extract of *T. glaberrima* upon chromatography on alumina gave two non polar fractions viz., a sterol and a terpenoidal. Sterol fraction upon GLC was found to contain spinasterol, stigmasterol and stigmasto-7-enol. On the other hand the terpenoidal fraction gave betulin upon crystallization in petroleum ether (Chawla et al., 1976). A triterpenoid viz., cycloart-23-ene-3β, 25-diol was isolated from the petroleum ether extract of *T. glaberrima* (Singh et al., 1978).

Two homologous series, one alkanes (C_{31}-C_{35}) and the other of wax esters (C_{52}-C_{56}), stigmast-22-en-3-ol and quercetin-3-rutinoside were isolated from the aerial parts of *T. glaberrima* (Manerikar and Kulkarni, 1978). Two sesquiterpene lactones were isolated from the chloroform extract of the aerial parts of *T. glaberrima*. The one with less polar was found to be Cynaropicrin and the other with more polarity was found to be 11, 13-dihydrodesacylcynaropicrin (Singhal et al., 1982).

A potential insect feeding deterrent compound viz., Cynaropicrin was obtained from the chloroform extract of the leaves of *T. glaberrima* (Bhattacharya et al., 1995).

1.5 Research envisaged

The purpose of present studies was, first to develop the methods for standardization of selected plants and thereby to provide a means for true identification of the individual plants and secondly to evaluate the plants for their reported biological activity and thereby justifying their role in therapeutics. The detailed steps for the studies were planned as follows:

- ✓ Collection and identification of the plant materials.
- ✓ Preparation of the extracts and Preliminary phytochemical studies
- ✓ Evaluation of macroscopic and microscopic characters, physicochemical constants, fluorescence behaviors, etc., for the selected plant materials.
- ✓ To develop the characteristic HPTLC finger print profiles for the selected plant materials.
- ✓ Determination of in-organic elements including the heavy metal content in the selected plant materials.
- ✓ Isolation and characterization of the compounds as markers.
- ✓ Development of HPTLC method for quality control of the selected plant materials using a suitable marker.
- ✓ Isolation of genomic DNA and its finger printing for the selected plant materials.
- ✓ Evaluation of the effect of extracts on male reproductive parameters using suitable animal models and thereby to standardize them pharmacologically.

REFERENCES

1. Abraham Z, Bhakuni SD, Garg HS, Goel AK, Mehrotra BN, Patnaik GK, 1986. Screening of Indian plants for biological activity Part XII, *Indian Journal of Experimental Biology* 24: 48-68.
2. Agarwal S and Chauhan S, Mathur R, 1986. Antifertility effect of emblin in male rats. *Andrologia* 18: 125-131.
3. Anonymous, 1952. The wealth of India, Raw Materials, vol. 3. CSIR, New Delhi, p.127.
4. Anonymous, 1976. The wealth of India, Raw Materials, vol. 10. CSIR, New Delhi, p.286.
5. Anonymous, 1978. Declaration of Alma-Ata: International conference on primary health care, Alma-Ata, USSR, 6-12 September.
6. Anonymous, 2000. The useful plants of India. NISCOM, New Delhi. pp 97-668.
7. Ayurvedic Pharmacopoeia of India, 1999. Goverment of India, ministry of heallth and family welfare. Department of Indian system of medicine and Homeopathy, New Delhi.
8. Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremmer WJ, Matsumoto AM, 1996. Combined administration of levonorgestral and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. *Journal of Clinical Endocrinology and Metabolism* 81: 757-762.
9. Bedi SJ, 1978. Ethnobotany of the Ratan Mahal Hills, Gujarat, India, *Economic Botany* 32: 278-284.
10. Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E, 1995. Potential of testosterone buciclate for male contraception: endocrine differences between responders and non-responders. *Journal of Clinical Endocrinology and Metabolism* 80: 2394-2403.
11. Bhatnagar LS, Singh VK, Pandey GJ, 1973. *Journal of Research in Indian Medicine* 8: 67-100.

12. Bhattacharya PR, Barua NC, Ghosh AC, 1995. Cynaropicrin from Tricholepis glaberrima: a potential insect feeding deterrent compound. *Industrial Crops and Products* 4: 291-294.
13. Bhiwagade DA, Averi KM, Menon HN, 1991. Effect of depot medroxy acetate on testis on testis of albino rats: Ultrastructural and biochemical studies. *Indian Journal of Experimental Biology* 29: 319-326.
14. BHP, 1983. British Herbal Pharmacopoeia: British herbal medicine association scientific committee. 24.
15. Bhutani KK, 2000. Finger printing of Ayurvedic drugs. *The Eastern Pharmacist* 43: 21-26.
16. Chaturvedi M, Mali PC, Dixit VP, 1995. Antifertility effects of the roots of *Echinops echinatus* (Roxb) in male rats. *Journal of Environment and pollution* 2(4): 153-157.
17. Chaudhary PK, 1987. Echinozolinone an alkaloid from *Echinops echinatus*. Phytochemistry 26(2): 587-589.
18. Chaudhary PK, 1988. Constituents of the flowers of *Echinops echinatus*. *Fitoterapia* 59(2): 150-151.
19. Chaudhary PK, 1992. 7-Hydroxyechinozolinone, A new alkaloid from the flowers of *Echinops echinatus*. *Journal of Natural Products* 55(2): 249-250.
20. Chaudhary PK, Thakur RS, 1986. An acylated flavone Apigenin 7-*O*-β-D-(4''-cis-*p*-coumaroyl) glucoside from *Echinops echinatus*. *Phytochemistry* 25(7): 1770-1771.
21. Chawla AS, Kapoor VK, Sangal PK, Gupta AK, Evans FJ, 1976. Chemical constituents of Tricholepis glaberrima. *Planta medica* 30: 151-153.
22. Chopra RN, Chopra IC, Handa KL, Kapur LD, 1994. Indigenous drugs of India, 2nd ed. Academic publishers, Culcutta, 180-674.
23. Chopra RN, Nayar SL, Chopra IC, 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi, p 247.
24. Cox PA, 1994. The ethnobotanical approach to drug discovery: strengths and limitations. In: Ethnobotany and the search for new

drugs. Ciba Foundation Symposium, 184, Chichester, John Wiley and Sons, 25-41pp.

25. Dixit VP, Agarwal M, Verma M, 1981. Male contraception properties of new synthetic steroid derivative (Danzol) in Rattus rattus rufescens. Endokrinologie 77: 30-38.
26. Dobriyal RM, Narayan DBA, 1998. Ayurvedic herbal raw material. *The Eastern Pharmacist* 41: 31-35.
27. Du XM, Kohinata T, Guo YT, Kazumoto M, 1998. Components of the ether-insoluble resin glycoside-like fraction from *Cuscuta chinensis*. *Phytochemistry* 48: 843–850.
28. Gaedeke J, Fels LM, Bokemeyer C, Mengs U, Stoltel H, Lentzen H, 1996. Cisplatin nephrotoxicity and protection by silibinin. *Nephrology Dialysis Transplantation* 11: 55-62.
29. Gauthaman K, Adaikan PG, Prasad RNV, 2002. Aphrodisiac properties of Tribulus terrestris extract in normal and castrated arts. *Life Sciences* 71: 1385-1396.
30. Gian S, Tapan M, 1998. Herbal Aphrodisiacs: A Review, *Indian drugs* 35(4): 175-182.
31. Griffin PD, 1989. Plants for fertility regulation. In: Research in human reproduction, WHO Biennial report 1986-87, WHO Task force on Human reproduction, Geneva, pp. 229-239.
32. Gupta SS, 1994. Prospects and perspectives of natural products in medicine, *Indian Journal of Pharmacology*, 26: 1-12.
33. Handa SS, 1995. Herbal raw materials and traditional remedies. *The Eastern Pharmacist* 38: 23-27.
34. Hang-Jun Pan, Hong-Xiang Sun, Yuan-Jiang Pan, 2005. Adjuvant effect of ethanol extract of Semen cuscutae on the immune responses to ovalbumin in mice. *Journal of Ethnopharmacology* 99: 99–103.
35. Harnischfeger G, 2005. Standardization and Quality Control of Medicinal Plants and their Products. UNIDO-ICS Training Course on: Development, quality assurance and regulatory aspects of herbal medicinal products; Panama City, Panama 21-25 November 2005.

36. Hogale MB, Uthale AC, 1988. Chemical constituents of *Echinops echinatus*. *Orient Journal of Chemistry* 4(3): 334-335.
37. ICS-UNIDO, 2004. Regional workshop on: Quality control of medicinal plant products in South East Asia. International centre for science and high technology – United nations Industrial Development organization, 23-24 july 2004, Kuala Lumpur, Malasiya.
38. IHP, 1998. Indian Herbal Pharmacopoeia I and II. Regional Research Laboratory, Jammu-Tawi and IDMA, Mumbai, INDIA.
39. Issar RK, 1974. The botanical identification of market sample of *Brahmadandi*. *Journal of Research in Indian Medicine* 9(1): 92-93.
40. Jain A, Katewa SS, Chaudhary BL, Galav P, 2004. Folk herbal medicine used in birth control and sexual diseases by tribals of southern Rajasthan India. *Journal of Ethnopharmacology* 90: 171-177.
41. Joshi D, Nagaraju V, 1991. *Indian Medicine* 3: 35-39.
42. Kakrani HK, Saluja AK, 1993. Traditional treatment through herbal drugs in kutch district, Gujarat state India. Part I Uterine disorders. *Fitoterapia* 65(5): 463-465.
43. Kakrani HN, Kakrani BH, Saluja AK, 2005. Traditional treatment of diabetes through herbs in kutch district, Gujarat state. *Planta indica* 1: 16-21.
44. Kamal R, Gupta RS, Lohiya NK, 2003. Plants for male fertility regulation. *Phytotherapy Research* 17: 579-590.
45. Kasturi M, Manivannan B, Ahmed N, Shaik PD, Pathan KM, 1995. Changes in epididymal structure and function of albino rats treated with Azadirachta indica leaves. *Indian Journal of Experimental Biology* 33: 725-729.
46. Khan MJ, Niazi HA, Khalid S, 2000. Ethnobotany and taxanomic studies of *Echinops echinatus* Roxb (Untkatara) from potohar region of Pakistan. *Hamdard medicus* 43(3): 61-64.
47. Kirtikar KR, Basu BD, 1975. Indian Medicinal Plants vol. 2. Periodical experts, Delhi, pp. 1415-1426.

48. Kulshreshtha SS, Mathur RS, 1990. Effect of steroidal fraction of seeds of Abrus precatorious Linn. On rat testis. *Indian Journal of Experimental Biology* 28: 752-756.
49. Lohiya NK, Manivannan B, Mishra PK, et al., 2002. Chloroform extract of Carica papaya seeds induces long term reversible azoospermia in langur monkey. *Asian Journal of Andrology* 4: 17-26.
50. Lue Y, Sinha Hakim AP, Wang C et al., 1998. Triptolide: a potential male contraceptive. *Journal of Andrology* 19: 479-486.
51. Manerikar SV, Kulkarni AB, 1978. Chemical investigation of *Tricholepis glaberrima. Indian Journal of Chemistry, Section B* 16: 439-440.
52. Misra B, 1969. Bhava Prakash Nighantu, commentary by K.C.Chunekar, Ed. G.S.Pandey, Chowkamba Vidya Bhavan, Varanasi, India. pp. 986.
53. Nadkarni AK, 1976. Indian Materia Medica vol. 1. Bombay Popular Prakashan Pvt. Ltd, Bombay, p. 468.
54. Nagata S, Kurosawa M, Mima K et al., 1999. Effect of anabolic steroid (19-nortestosterone) on the secretion of testicular hormone in the stallion. Journal of Reproduction and Fertility 115: 373-379.
55. Oudhia P 2003a. Untkatara as Medicinal Herb in Chhattisgarh, India: Traditinal Medicinal Knowledge, Occurrence and Trade. http://botanical.com/site/column_poudhia/250_brahmadandi.html
56. Oudhia P. 2003b. Medicinal Herbs of Chhattisgarh, India having less known uses of. XXXIV. Brahmadandi (*Tricholepis glaberrima*). http://botanical.com/site/column_poudhia/250_brahmadandi.html.
57. Patwardhan B, Kalbag D, Patki PS, Nagasampagi BA, 1990. Search of immunoregulatory agents-a review. *Indian Drugs* 28: 56–63.
58. Purandare TV, Kholkute SD, Gurjar A et al, 1979. Semen analysis and hormonal levels of bonnet Macaques administered *Emblia ribes* berries, an indigenous plant having contraceptive activity. *Indian Journal of Experimental Biology* 17: 935-936.
59. Puri HS, Quart J, 1971. *Journal of crude drug research* 11: 1742-1743.

60. Pushpangadan P, 1995. Role of traditional medicine in primary healthcare. In: Iyengar PK, Damodaran VK, Pushpagadan P. Science for health. State committee on science technology and environment, government of kerala, INDIA.
61. Qian SZ, 1987. Tripterygium wilfordii: A Chinese herb effective in male fertility regulation. *Contraception* 36: 247-263.
62. Rajani M, Padh H, 2000. Fourth international symposium on innovations in pharmaceutical sciences and technology, Ahmedabad, India.
63. Ram SN, Roy R, Singh B, Singh RP, Pandey VB, 1996. An acylflavone glucoside of *Echinops echinatus*. *Planta Medica* 62(2): 187.
64. Rao MV, Ray GK, 1993. Biochemical and morphological changes of spermatozoa in progestin androgen injected rats. *Indian Journal of Experimental Biology* 31: 12-15.
65. Reddy MB, Reddy KR, Reddy MN, 1989. A survey of plant crude drugs of Anantpur District of Andhra Pradesh, India. *International Journal of Crude Drug Research* 27(3): 145-155.
66. Savitasharma K, Mehta BK, 1989. Antimicrobial activity of *Echinops echinatus* root extracts. *Fitoterapia* 60: 82-83.
67. Savitasharma K, Mishra S, Mehta BK, 1988. Antifertility activity of *Echinops echinatus* in albino rats. *Indian Journal of Medical Sciences*, 42: 23-26.
68. Scambia G, De Vincenzo R, Ranelletti PO, Benedetti Panici P, Ferrandina G, D'Agostino G, Fattorossi A, Bombardelli E, Mancuso S, 1996. Antiproliferative effect of silybin on gynaecological malignancies: synergism with cisplatin and doxorubicin. *Europian Journal of Cancer* 32(5): 877-882.
69. Sen AB, Dhar MM, Shukla YN, 1968. Chemical examination of *Echinops echinatus*. *Journal of Indian Chemical Society* 45(8): 697-698.

70. Shah GL, Gopal GV, 1985. Ethnomedical notes from the tribal inhabitants of the north Gujarat (India). *Journal of Economic and Taxanomic Botany* 6(1): 193-201.

71. Shah MJ, Subhan F, Tahir F, Alam W, 1997. Male contraceptives from traditional drugs (plant based). *Hamdard Medicus* 40(2): 34-36.

72. Shah SA, Ravishankara MN, Nirmal A, Shishoo CJ, Rathod IS, Suhagia BN, 2000. Estimation of individual sennosides in plant materials and marketed formulations by an HPTLC method. *Jornal of Pharmacy and Pharmacology* 52: 445-449.

73. Shaik PD, Manivannan B, Pathan KM, Kasturi M, Ahmed N, 1993. Antispermatic activity of Azadirachta indica leaves in albino rats. *Current Science* 64: 688-689.

74. Sharma S, Porwal M, Mehta BK, 1988. Chemical investigation of *Echinops echinatus*. *Journal of Indian Chemical Society* 65(1): 69-70.

75. Sikarwar RLS, Kaushik JP, 1993. Folk medicine of the morena district, Madhya Paradesh, India. *International journal of pharmacognosy* 31(4): 283-287.

76. Sing A, Kapoor VK, Sangal PK, 1978. Cycloart-23-ene-3β, 25-diol from *Tricholepis glaberrima*. *Planta Medica* 34: 109-110.

77. Singh B, Gambhir SS, Pandey VB, Joshi VK, 1989. Anti inflammatory activity of *E. echinatus*. *Journal of Ethnopharmacology* 25: 189-199.

78. Singh B, Ram SN, Pandey VB, Joshi VK, Gambhir SS, 1991. Studies on anti-inflammatory activities of Taraxasterol acetate from *Echinops echinatus* in rats and mice. *Phytotherapy Research* 5: 103-106

79. Singh KK, Maheshwari JK, 1994. Traditional Phytotherapy of some medicinal plants used by the Tharus of the Nainithal District of Uttar Pradesh, India. *International journal of pharmacognosy* 32(1): 51-58.

80. Singh KN, Pandey VB, 1989. Constituents of *Echinops echinatus*. *Orient Journal of Chemistry* 5(4): 342-344.

81. Singh S, Upadhyay RK, Pandey MB, Singh JP, Pandey VB, 2006. Flavonoids from *Echinops echinatus*. *Journal of Asian Natural Products Research* 8(3): 197–200.

82. Singh UP, Pandey VB, Singh KN, Singh RDN, 1988. Antifungal activity of some new flavones and flavone glycosides of *Echinops echinatus*. *Canadian Journal of Botany* 66(9): 1901-1903.

83. Singh VK, Ali ZA, 1992. A contribution to the Ethnopharmacological study of the udaipur forests of Rajasthan, India. *Fitoterapia* 63(2): 136-144.

84. Singhal AK, Chowdhury PK, Sharma RP, Baruah JN, Herz W, 1982. Guaianolides from *Tricholepis glaberrima*. *Phytochemistry* 21: 462-463.

85. Subramoniam A, Madhavachandran V, Rajasekharan S, Pushpangadan P, 1997. Aphrodisiac activity of *Trichopus zeylanicus* extract in male mice. *Journal of Ethnopharmacology* 57: 21-27.

86. Sudhakar S, Rao RS, 1985. *Journal of Economic and Taxanomic Botany*, 7: 399-406.

87. Thakur MJ, Mishra IN, Chaudhary BK, 1992. *Journal of Economic and Taxanomic Botany* 16: 383-390.

88. Udoh P, Kehinde A, 1999. Studies on the antifertility effect of paw paw seeds (Carica papaya) on the gonads of male albino rats. *Phytotherapy Research* 13: 226-228.

89. USP, 1990. The United States Pharmacopoeia, The United States Pharmacopoeial Convention, Inc.

90. Vaidya B, 1982. Some controversial drugs in Indian medicine, Chaukhambha orientalia, Varanasi, India, 314-315.

91. Varma R, 1982. *Journal of Economic and Taxanomic Botany* 3: 689-694.

92. WHO Task Force on Methods for the Regulation of Male Fertility, 1996. Contraceptive efficacy of testosterone induced azoospermia and oligozoospermia in normal men. *Fertility and Sterility* 65: 821-829.

93. WHO, 1991. Guidelines for the Assessment of Herbal Medicines. World Health Organization, Geneva, (WHO/TRM/91.4).

94. WHO, 2002. Traditional medicine: Report by Secretariat, EB111/9, 12 December 2002.

95. Wu FCW, 1997. Androgen/progestin combination in male contracption: Efficacy and safety. In proceeding of VIth international congress of Andrology. *Salzgurg* (Austria) may 25-29, 221-226.

96. Wu FCW, Farley TMM, Perogoudov A, Waites GMH, 1996. WHO Task Force on the Methods for the Regulation of Male Fertility. Effect of testosterone enanthate in normal man experience from multi-centre contraceptive efficacy study. Fertility and Sterility 65: 626-636.

97. Xiang SH, Jun PH, Jiang PY, 2003. Haemolytic activities and immunologic adjuvant effect of *Panax notoginseng* saponins. *Acta Pharmacologica Sinica* 24 (11): 1150-1154.

98. Xiaorui Zhang, 1998. Regulatory Situation of Herbal Medicines A Worldwide Review, WHO/TRM/98.1.

99. Xiong YB, Zhou CH, 1994. The effect of extracts from herba epimedii and semen cuscutae on the function of male reproduction. *Chinese Pharmaceutical Journal* 29: 89–91.