CHAPTER 2

REVIEW OF LITERATURE

Wild plants play an important role in rural communities, as the natives mostly depend on plants for food, traditional medicine and other livelihood requirements. The use of edible wild plants forms a part of the cultural traditions of rural and suburban societies throughout the world. In recent years, local knowledge has been increasingly studied using various ethnobotanical tools to identify plants with high nutritional, medical and commercial potentials which are likely to contribute in better living of rural population. Among wild plants, some *Calamus sp.* commonly known as Rattans are reported to have disease healing ability and are edible. *Calamus tenuis* Roxb., one of the edible Rattan is traditionally used by the natives of Dibrugarh district of Assam to cure certain health ailments and its tender shoots are eaten in the form of vegetables and in other culinary preparations.

In this perspective the present study was designed under the title **"Consumption pattern of *Calamus tenuis* Roxb. shoots of the forest village natives of Dibrugarh, Assam and investigation of its cytotoxicity activity on cancer and normal cells (A549, MCF7 and L132)"**. This chapter compiles the available review of literature for the study in to following sections.

2.1 Indigenous wild edible plants

- 2.1.1 Importance of indigenous wild edible plants
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- 2.1.3 Nutritional prospect of wild edible plants (WEPs)
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- 2.1.5 Medicinal properties of indigenous wild edible plants
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2.1 Indigenous wild edible plants

2.1.1 Importance of indigenous wild edible plants

Indigenous wild edible plants refer to species that are neither cultivated nor domesticated, but are available from wild natural habitat and used as sources of food [Beluhan and Ranogajec, 2010]. The use of wild edible plants (WEPs) was present in historic and contemporaneous rural and indigenous societies globally [Vincent, 1985; Hladik and Hladik, 1990; Bahuchet *et al.*, 1991; Maneenoon *et al.*, 2008; Swarnkar and Katewa, 2008; Gemedo-Dalle *et al.*, 2005]. When compared to domesticated plant food sources, wild plant foods tend to be overlooked. However there is substantial evidence that indicates the importance of wild edibles in terms of the global food basket. Since WEPs are freely accessible within natural habitats, indigenous people have knowledge of how to gather and prepare the food from wild sources [Somnasang and Moreno-Black, 2000]. According to Abermound (2009), about one billion people in the world uses wild foods (mostly from plants) on a daily basis. Besides, over 300 million people obtain a considerable part of their livelihood in the form of Non-Timber Forest Products (NTFPs) from wild forests [Belcher *et al.*, 2005].

2.1.2 Contribution of wild edible plants (WEPs) to food security

According to Jaenicke and Hoschle-Zeledon (2006), over 50 percent of the world's daily requirement of proteins and calories is obtained from only three crops: wheat, maize and rice. The dependence on a few domesticated species limits dietetic diversity and leads to over dependence on limited resources. By contrast, ethnobotanical investigations on WEPs suggest that more than 7,000 species have been used for food in human history [Grivetti and Ogle, 2000]. In countries such as China, India, Thailand and Bangladesh; hundreds of WEPs are still consumed along with domesticated species of plants [Mazhar *et al.*, 2007]. The document of Rathore

(2009) shows the presence of 600 WEP species in India. Moreover, Boa (2004) documented the presence of over 1000 species of wild edible fungi (which do not belong to the plant kingdom but are closely related to it) worldwide. These figures show the intimate link between WEPs and the diets of many people, hence wild edibles can be considered to be useful resources in the efforts to achieve food security. Further to their roles in food security, many WEPs such as *Adansonia digitata* L. (Malvaceae), *Moringa stenopetala* (Bak. f.) Cufod. (Moringaceae), *Syzygium guineense* (Willd.) DC. (Myrtaceae) and *Ximenia americana* L. (Olacaceae) are acknowledged for their medicinal, cultural, forage and economic values [Johns *et al.*, 1996; Ogle *et al.*, 2003; Reyes-Garcia *et al.*, 2005; Shrestha and Dhillon, 2006].

2.1.3 Nutritional prospect of wild edible plants (WEPs)

Leaves, stems, fruits, flowers, tubers, barks, seeds, roots, and so on, of lots of WEPs are still consumed for their dietary value in many communities around the globe. Some of these WEPs are used as primary food sources while others are used as secondary condiments in dishes prepared from domesticated cultivars [Lockett and Grivetti, 2000]. These plants play an important role as a source of energy and micronutrients [Afolayan and Jimoh, 2009; De Caluwe, 2010a and 2010b]. Currently, preliminary research results on dietary analysis of many WEPs provide promising information (Table 2.1.3).

Table 2.1.3: Nutrition analysis reports on WEP's in some countries

No	WEP species	Family	Nutritional value	Region	Source
1	Abrus precatorious L.	Fabaceae	Protein, Ca, Fe, K, Na, Mg, Mn, Zn	Cameroun	Glew *et al.*, 2010
2	Burnatia enneandra Micheli	Alismataceae	Protein, Ca, Fe, K, Na, Mg, Mn, Zn	Cameroun	
3	Cadaba farinose	Capparidaceae	Protein, fat, Ca, Fe, K, Na, Mg, Mn, Zn	Cameroun	
4	Agave salmiana Otto	Agavaceae	Protein	Mexico	Lopez-Garcia and Basurto, 2007
5	Aloe vera L.	Aloaceae	Protein	Mexico	
6	Arbutus xalapensis Kunth	Ericaceae	Protein	Mexico	
7	Erythrina americana Mill.	Fabaceae	Protein	Mexico	
8	Euphorbia radians Benth.	Euphorbiaceae	Protein	Mexico	
9	Yucca filifera Chabaud	Agavaceae	Protein	Mexico	
10	Ximenia Americana L.	Olacaceae	Ca, P, Mg	Niger	Freiberger *et al.,* 1998
11	Amaranthus viridis L.	Amaranthaceae	Protein, Ca, Fe, P	Niger	
12	Corchorus tridens L.	Tiliaceae	Protein, fat, P, Cu	Niger	
13	Hibiscus sabdariffa L.	Malvaceae	Protein, fat, P	Niger	
14	Maerua Crassifolia Forssk	Capparidaceae	Fat, Ca, P	Niger	
15	Moringa oleifera Lam.	Moringaceae	Protein, fat, P	Niger	
16	Leptadenia hastata Decne	Asclepiadaceae	Protein, fat, Ca, P	Niger	
17	Borassus aethiopum Mart.	Aracaceae	Protein, Zn	Sahel region	Glew *et al.*, 2005
18	Tamarindus indica L.	Fabaceae	Carbohydrate, protein, fat	Sahel region	
19	Portulaca oleracea L.	Portulacaceae	Protein, fat	India, Iran	Aberoumand and Deokule, 2009
20	Asparagus officinalis L.	Asparagaceae	Protein, fat	India, Iran	

In addition, Becker (1983) reported the presence of vitamins A, B2, and C in WEPs of Senegal. Research on six WEPs from Spain also confirmed the occurrence of lipids,

fatty acids and carotenes in the leaves of these species [Guill-Guerrero and Rodriguez-Garcia, 1999]. Protein content in a proportion that is comparable to the amount in domesticated plants was also reported from a nutritional study of WEPs in South Africa [Afolayan and Jimoh, 2009]. A study on the dietary value of eight wild edibles in Iran and India also showed the presence of sodium, calcium, potassium, iron, zinc, protein, and fat in a ratio comparable to that found in cultivated plants [Aberoumand, 2009]. Many wild leafy vegetables of Poland are also mentioned for their rich content of vitamin C, natural antioxidants, carotenoids and folic acid [Luczaj, 2010]. Thus, the information available from the nutritional analysis of WEPs gives us an idea about their potential contribution to dietetic diversity and food security.

2.1.4 Role of cultural and socio economic status (SES) on consumption and other uses of indigenous plants

Wild edible plants play a critical role in ensuring food and livelihood security for countless families and communities around the world [Bell, 1995; Guijt *et al*., 1995; Lulekal *et al*., 2011; Neudeck *et al*., 2012; Teketay *et al*., 2010]. They have significant role in bridging the gap between food and energy supply, particularly to poor members of the community [Asfaw and Tadesse, 2001; Addis *et al*., 2005; Teketay *et al*., 2010].

The subsistence production of the rural population comprises fishery, agriculture, livestock husbandry, and the collection of forest resources. Farmers and others livelihoods and economic development is hampered by a low level of education, limited income alternatives and poor infrastructure. Collection of forest products provides an important supplementary source of income [SuLaMa, 2011]. The intensity of local usage of WEPs depends on taste, local needs, market prices, location

and harvested amounts. Other factors governing its uses are differences in culture, gender, language, ethnicity, political belief system, personal preferences, appropriation skills and the availability of these resources in collection areas [Bardhan and Dayton-Johnson, 2000]. The collection of quantities and qualities of plants vary greatly between households. The poor population consume and sale more WEPs and have higher knowledge on traditional usages of medicinal plants than that of rich individuals [Andriamparany *et al.*, 2014]. Households with more cropland and higher crop harvest, collect less forest products [Reddy and Chakravarty, 1999]. An overall household's wealth status affects the traditional knowledge and usage intensity of forest products [Beltran-Rodriguez *et al.,* 2014; Khanal, 2001; Sapkota and Oden, 2008]. The World Resources Institute (2005) reported that families facing poverty, sickness, drought, wars and economic crisis depend to a higher degree on the collection of wild resources.

Socio-cultural factors are of higher importance for the use of medicinally important wild plants. The uses of forest products are significantly higher in villages near forests, where wild and medicinal plants are more readily available [Banana and Turiho-Habwe, 1997]. Furthermore, poor market access may increase the importance of forest products to sustain people's livelihood [Gunatilake, 1998]. The rate of change in social and economic attributes of rural households is likely proportional to the rate of change in resource use [Kant, 2000]. Therefore, whatsoever the products extracted, a household's socio-economic dynamics ultimately drives its ability to use the forest resources.

2.1.5 Medicinal properties of indigenous wild edible plants

About 80% people of the world, particularly in the rural areas of developing countries, continue using traditional resources in healthcare [Poonamand and Singh, 2009]. Plants have been always considered as a primary source of drugs in traditional and alternative system of medicine in various forms such as crude form, juice, decoction, and crude extracts. [Poonamand and Singh, 2009]. Out of the total 4,20,000 flowering plants reported globally [Govaerts, 2001], more than 50,000 are used for medicinal purposes [Schippmann *et al*., 2002].

Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. Medicinal plants play an important role in the development of potent therapeutic agents. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. More than 64 plants have been found to possess significant antibacterial properties; and more than 24 plants have been found to possess antidiabetic properties. *Daboia russellii* and *Naja kaouthia* are used for antidote activity. Lupeol acetate isolated from the root extract of Indian sarsaparilla Hemidesmus indicus neutralizes venom [Chatterjee *et al.*, 2006].

In India, there are about 54 million indigenous people of different ethnic groups inhabiting various terrains. These indigenous groups possess their own distinct culture, religious rights, food habit and have a rich knowledge of traditional medicine [John, 1984; Pushpangadan and Atal, 1984; Anuradha *et al.*, 1986; Harsha *et al.*, 2002; Parinitha *et al.*, 2005]. The indigenous community use rhizome paste of plants like *Acorus calamus*, stem bark of *Bunchania lanzan*, stem and leaves of *Moringa oleifera*, *Achyrnthus aspera*, *Gynandropsis gynandra*, *Bombax ceiba* as antidote of snake bite and scorpion sting. They prepare paste from powdered root, stem and

leaves of some plants like *Vanda tessala, Alternanthera sessiles* and of roots of *Cassia adnata, Sida cordata,Bauhina purpurea* etc. and apply on broken bone portions and tie for 10-15 days for healing of bone. They also use plants like *Equisetum ramosissimum, Argemone maxicana* in dried, powdered and paste form on infested portion of skin and on wounds. Plants like *Bauhinia purpurea, Sida acuta, Jatropha curcus, Grewia hirsutum, Albizzia lebbeck, Capparis decidu*as are used in muscular pain, cure of fever, headache and body swelling [Rai and Nath, 2016].

India is a vast repository of medicinal plants that are used in traditional medical treatments [Chopra *et al.,* 1956]. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments [Rabe and Staden, 1997]. The use of herbal medicine is gaining popularity due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures [Agarwal, 2005]. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health [Sane, 2002].

Recently herbal health care sector is gaining impetus due to the shift in the mindset of people towards herbal medicines which are considered safe and having wider biological activity [Sharma *et al.,* 2008]. More than 43% of the total flowering plants in India are reported to be of medicinal importance [Pushpangdan, 1995]. Growing demand for plant based medicines and health care products have led to an increase in the number of herbal drug manufacturers. With around 25,000 licensed herbal drug manufacturers in India it has become a lucrative business with a global market currently standing over $ 60 billion annually and is expected to get higher at 6.4%

average growth rate. In Indian herbal industry, about 1000 single drugs and 3000 compound preparations have been registered so far. Except few plant species like *Opium, Senna, Psyllium, Periwrinkle, Cinchona* etc., which are obtained from commercial cultivation, majority of other plant species are collected from the natural sources [Inamdar *et al.,* 2008, Verma and Singh, 2008; Kamboj, 2000].

2.1.6 Commercialization of herbal medicine

The market for ayurvedic medicines is estimated to be expanding by 20% annually. Sales of medicinal plants have grown by nearly 25% in India in past ten years (1987-96), the highest rate of growth in the world [Masood, 1997]. But the per capita expenditure in India on medicines per annum is amongst the lowest in the world. In other developing countries too, plants are the main source of medicine. Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine uses over 5000 plant species; India uses about 7000. According to Export Import Bank, the international market for medicinal plant related trade having a growth rate of 7% per annum. China's share in world herbal market is US$ 6 billion while India's share is only US$1 billion. The annual export of medicinal plants from India is valued at Rs. 1200 million. All the major herbal-based pharmaceutical companies are showing a constant growth of about 15 per cent.

Recently even developed countries, are using medicinal systems that involve the use of herbal drugs and remedies. Undoubtedly the demand for plant derived products has increased worldwide. The demand is estimated to grow in the years to come fuelled by the growth of sales of herbal supplements and remedies. This means that scientists, doctors and pharmaceutical companies will be looking at countries like China, India,

etc. for their requirements, as they have the most number of medicinal plant species and are the top exporters of medicinal plants.

2.1.7 Efficacy and safety issues of herbal medicine

Over the past decade, the use of herbal medicines represents approximately 40% of all healthcare services delivered in China while the percentage of the population which has used herbal medicines at least once in Australia, Canada, USA, Belgium, and France is estimated at 48%, 70%, 42%, 38%, and 75%, respectively [Foster *et al.*, 2000; WHO, 2002b]. In spite of the positive perception of patients on the use of herbal medicines and alleged satisfaction with therapeutic outcomes coupled with their disappointment with conventional allopathic or orthodox medicines in terms of effectiveness and/or safety [Huxtable, 1990; Abbot and Ernst, 1997], the problem of safety of herbal remedies continues to remain a major issue of concern.

It has been observed that most of the problems associated with the use of traditional and herbal medicines arise mainly from the classification of many of these products as foods or dietary supplements in some countries. As such, evidence of quality, efficacy, and safety of these herbal medicines is not required before marketing. In the same vein, quality tests and production standards tend to be less rigorous or controlled and in some cases, traditional health practitioners may not be certified or licensed. The safety of traditional and herbal medicines has therefore become a major concern to both national health authorities and the general public [Kasilo and Trapsida, 2011].

There are further concerns over the perceived lack of adequate regulation of herbal medicines in many countries and the encouragement of the sale of unregistered products, which are not controlled by regulatory authorities [De-Smet, 1995]. The legal status of herbal medicines varies widely from country to country, and the

regulation of these medicines has not evolved in methodological manner [WHO-TRM, 1998]. Most countries have different ways of defining medicinal plants, herbs or the products derived from them and have adopted various approaches for licensing, dispensing, manufacturing and trading to ensure their quality, safety and efficacy [WHO-TRM, 1998]. The European Union (EU) implemented a directive to harmonize the regulation of traditional herbal medicine products across the EU and establish a simplified licensing system in order to help the public to make informed choices about the use of herbal products. This demanded all manufactured herbal products to either gain a product license of the type needed to manufacture "conventional" products or become registered as a "traditional herbal medicinal product" [Routledge, 2008; Raynor *et al.,* 2011]. It was made compulsory that they must accompany comprehensive information such as indications, precautions, how to use the product, side effects, how to store the product and regulatory information, for safe use. This information is usually provided on a leaflet inserted into the product package [Raynor *et al.,* 2011]. Thus in Europe, herbal medicines are well established and formally regulated, whereas in the United States, herbs are classified as dietary supplements and therapeutic claims are not allowed. The situation is even more precarious in many developing countries, where despite of the great number of traditionally used herbal medicines and much empirical knowledge about their use, there are very few legislative criteria that can be employed to incorporate traditionally used herbal medicines into national drug policies [WHO-TRM, 1998]. Thus, in both industrialized and developing nations, the assurance of quality, safety and efficacy has now become a key issue [WHO-TRM, 1999]. Therefore, a careful scientific evaluation of safety and efficacy of herbal medicines is essential before they can be officially incorporated into primary healthcare systems and global acceptance of their health benefits. A

centralized, focused approach to provide accurate and up-to-date scientific information on commonly used herbal medicines is required.

2.1.8 Interaction of herbs with drugs

The potential for interactions between medications and herbs is one of the significant consequences resulting from the use of several medications, herbal products and supplements. Unfortunately, many consumers of herbal products assume that because these products are from natural sources, they are safe [Izzo and Ernst, 2009]. However, there are a variety of case reports and clinical observations regarding the occurrence of clinically significant interactions between herbs and medications [Hu *et al.,* 2005]. There are other reports of interactions that are theoretically based on preclinical data [Hu *et al.,* 2005].

The mechanisms for these herb/drug interactions are not fully understood, but both pharmacokinetic and pharmacodynamic processes have been identified as playing a major role [Izzo and Ernst, 2009; Hu *et al.,* 2005]. In general, herbal products may mimic, increase, or decrease the effects of medications [Hu *et al.,* 2005]. It is possible that the herbal product itself has therapeutic properties that are synergistic or an additive to the medication being used. Examples of herbs that enhance the therapeutic effect of a medication include Ephedra used with amphetamines, valerian or Kava with benzodiazepines. This may lead to supratherapeutic effects or toxicities, complicating the management of medical conditions and the corresponding medications. Similarly, an herb may counteract the desired effect of a medication, as in use of Ephedra with antihypertensive medications. Pharmacokinetic drug interactions can lead to alterations in the absorption, distribution, metabolism and excretion of medications. Studies have revealed that these interactions occur through the induction or inhibition of drug metabolizing enzymes (cytochrome P 450) or

alteration of drug transporters (P-glycoproteins) [Hu *et al.,* 2005]. Herbal products that inhibit the metabolism of medications result in higher medication levels, which can increase efficacy or risk for toxicity. Herbs that induce metabolism of medications can lead to decreased medication levels, which may result in decreased efficacy of the medication or therapeutic failure. Herbs that cause p-glycoprotein alterations can have an impact on the absorption and bioavailability of the medication and either reduce or potentiate the effects [Evans, 2000; Ioannides, 2002; Zhou *et al.,* 2003].

2.2 Documentation of wild edible indigenous plants used for food and therapeutic purposes

2.2.1 Documentation of uses of indigenous plants as food

Wild edible plants (WEPs) have been a focus of research for many ethnobotanists in recent decades. Currently, there is renewed global interest in documenting ethnobotanical information on neglected wild edible food sources [Bharucha and Pretty, 2010]. Since traditional knowledge on WEPs is being eroded through acculturation and the loss of plant biodiversity along with indigenous people and their cultural background, promoting research on wild food plants is crucial in order to safeguard this information for future societies [Asfaw, 2009].

2.2.2 Documentation of indigenous plants uses for therapeutic purposes

Tribal healers in most of the countries, where ethnomedical treatment was frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provided instructions to local people as how to prepare medicine from herbs [Puspangadan and Atal, 1984; Perumal and Ignacimuthu, 1998]. They kept no records and the information was mainly passed on verbally from generation to generation [Dhar *et al.,* 1973]. World Health Organization (WHO) showed great interest in

documenting the use of medicinal plants used by tribals from different parts of the world [Kaido *et al.,* 1997]. Many developing countries had intensified their efforts in documenting the ethnomedicinal data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herbs had been intensified. In the last decade, many countries have given priority to the documenting of WEPs and the associated indigenous knowledge [Reyes-Garcia *et al.,* 2005; Tardio *et al.,* 2006; Rashid *et al.,* 2008] so that the people will be better informed regarding efficacious drug treatment and improved health status [Manandhar, 1987]. Documentation of traditional knowledge especially on the medicinal uses of plants has provided many important drugs of the modern day [Cox and Balick, 1994; Fabricant and Farnsworth, 2001]. However, even today this area holds much more hidden treasure.

Indian subcontinent is renowned for its cultural and plant biodiversity where large numbers of people are still living in tribes. These tribal people possess a pool of undisclosed, ethnomedicinal, and ethnopharmacological information regarding the flora of their surroundings, and which may prove to be very helpful in rural community with its advantage [Pandey and Shukla, 2003; Semwal, 2005]. Natural wealth as well as the undisclosed ethnopharmacological information and the tribal cultures have been decreased remarkably at a disturbing rate due to change in life style, unintentional developmental programs, and mounting recent civilization. Negligence by the youth also influences the traditional knowledge [Pandey and Shukla, 2003; Semwal, 2005]. Therefore, it is necessary to discover and document this exceptional, original, and conventional information of the ethnic population, before it disappears with the knowledgeable persons. It is also important for the establishment of these conventional principles at the national and international level realizing the recent global trends [Kala *et al.,* 2006].

2.3 Rattan *(Calamus sp.)*: Its uses and therapeutic properties

Young shoots of the plant are used as vegetable [Barua *et al.,* 2007]. In Ayurvedic system Calamus species are useful in fever, piles, dyspepsia and biliousness. Flowers are used externally as antiseptic and anti-bacterial for cuts, burns, bruises and scalds [Khare, 2007]. Roots, fruits and leaves are also used in folk medicine [Kumar and Sastry, 2014]. Also, some studies have revealed that a few edible species of rattan plant possesses anti-inflammatory activity as well as cell growth inhibitory effect against cancer cells. Dransfield and Manokaran (1994) specified that young rattan shoots are eaten and also used in local medicine. According to Boonsermsuk *et al.* (2007) and Sricharatchanya (2000), apart from use of rattan as an ingredient in many delicious and nutritious dishes and it have medicine properties. Takashi *et al.* (2005) reported that *Calamus insignis* has cytotoxicity property against HeLa cells and phytoconstituents of *C. insignis* exhibited cell growth inhibitory activity against HeLa cells even at very low concentration. According to Gracia *et al.* (2008), the treatment of *Calamus ornatus* tender shoots phytoconstituents against various cancer cell lines (MCF-7), CNS (SF-268), lung (NCI-H460), colon (HCT-116) and gastric (AGS) reduced cell proliferation and also the pure non-nutritive functional agents (NFAs) reduced the cell viability of breast, central nervous system (CNS), lung, colon and gastric cancer cell lines by 37.5%, 22.4%, 53.3%, 58.2%, 40.3% and 29.8%, 21.3%, 45.6%, 37.1%, 25.0% at concentrations of 24.2 and 21.2 uM respectively. They also recommended inclusion of rattan-shoots in diet for preventing inflammation and cancer-related illnesses. Campbell (2009) stated that rattan is an important non-timber forest product in Laos, being used in handicrafts and food source as well. Canes from mature plants are woven into many products and young shoots are a traditional part of the local diet. Rattan shoots are a popular food in Laos, traditionally harvested wild

from forests. However, population pressure, economic development and forest depletion have led to the establishment of rattan shoot plantations. Chang *et al.* (2010) studied the pharmacological effects of *Calamus quiquesetinervius* and found that an ethyl acetate extract of the stem exhibited notable antioxidant, anti-inflammatory and anti-platelet aggregation activities. Tag *et al.* (2012) found that *Calamus tenuis* has anti-diabetic effect on rodent models however no sufficient clinical evidence was found for its effectiveness. Borah *et al.* (2013) investigated *Calamus leptospadix* Griff. (Arecaceae) against helmintic disease and found that it possesses significant anthelmintic activity when compared with the normally used drug and hence provided a basis for the traditional practice of this plant by the people of Assam for treating intestinal worm infections. The screening of free radical scavenging potentiality of 84 medicinal plants including *Calamus tenuis* by Uddin *et al.* (2013) concluded that the plants having good scavenging property may have various health beneficial effects. They also reported the use of *Calamus tenuis* Roxb. as vegetables to be beneficial in diarrhea, edema and intrinsic hemorrhage. A pharmacological investigation on methanolic extract of *Calamus tenuis* Roxb. fruits for analgesic activity on mice derived that the extract decreased the dose dependent motor activity and exploratory behaviour of mice in Hole Cross and Open Field Test. The number of fields crossed in open field test and holes crossed in hole-cross test decreased as time passed. These results suggested that the extract possesses analgesic and CNS depressant activity [Hossain, 2013]. According to Ahmed *et al.* (2014), the fruit extracts of *Calamus tenuis* Roxb. too have antioxidant and cytotoxic activity. They also reported the presence of phytochemical constituents like alkaloid, flavonoids, tannin and steroid in the plant [Ahmed *et al.*, 2014].

2.4 *Calamus tenuis* Roxb.

2.4.1 Description of Calamus tenuis Roxb.

The plant is slender and has cluster forming climber stems upto 20 m long and 2.5 cm in diameter. Leaf sheaths are green with blackish brown straight spine measuring upto 2 cm which are scattered, flattened and densely arranged at sheath apices. Ocreas and flagella are present. Leaf rachis measure upto 1 m long with 31-42 linear leaflets arranged regularly per side gradually becoming smaller upward. Inflorescence measures upto 2.5 m long. Flowering branches are short and fruits are whitish or yellowish brown and globose or ellipsoid in shape which measure upto 1.6 cm long and 1.2 cm in diameter (Plates- 2.4.1.1, 2.4.1.2, 2.4.1.3, 2.4.1.4) [Henderson, 2009].

Plate-2.4.1.1: *Calamus tenuis* Roxb. in natural habitat

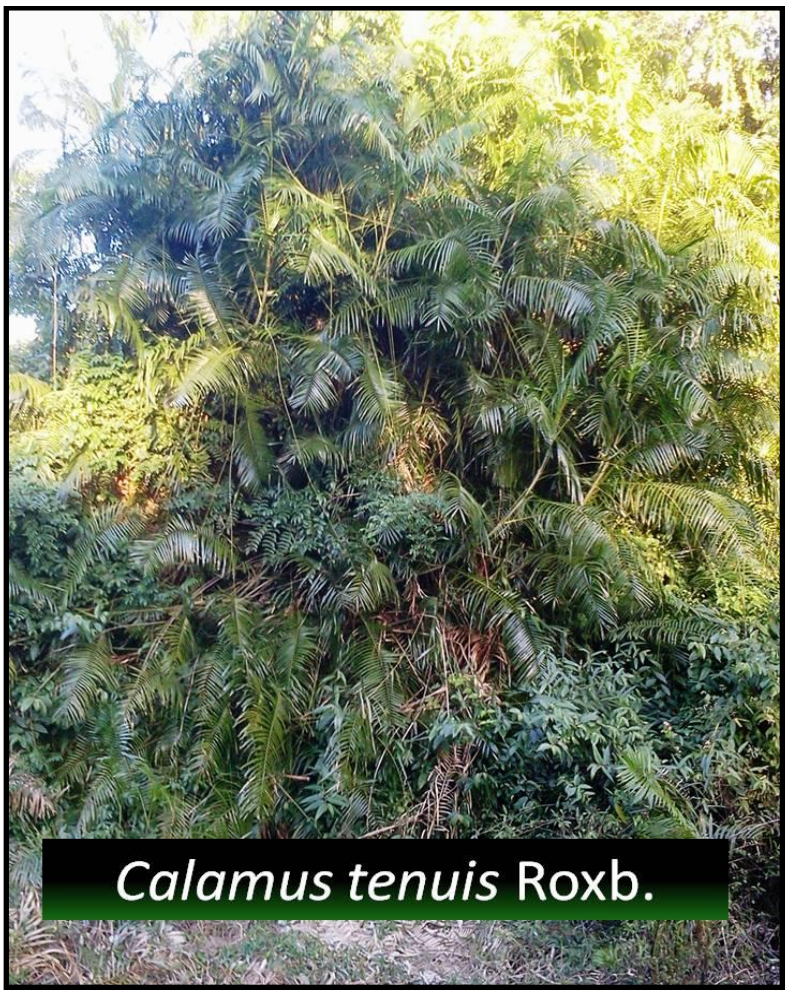


Plate-2.4.1.2: *Calamus tenuis* Roxb. fruit

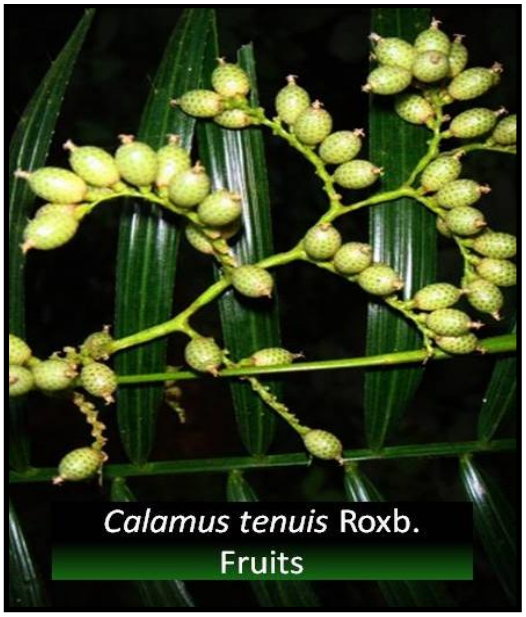


Plate-2.4.1.3: *Calamus tenuis* Roxb. inflorescence

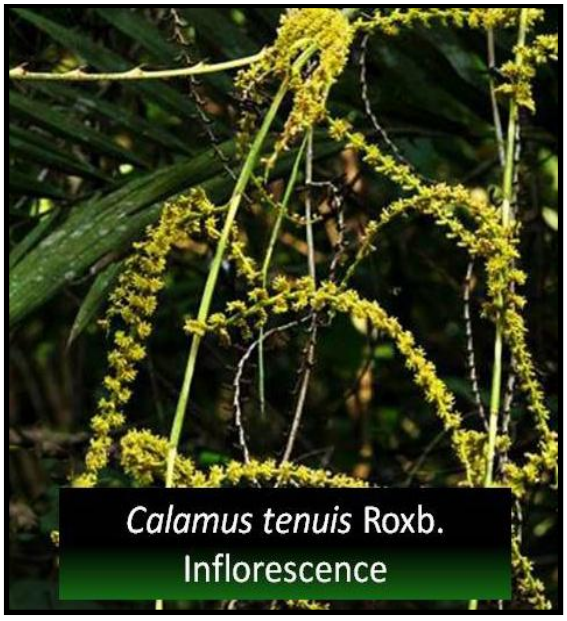


Plate-2.4.1.4: *Calamus tenuis* Roxb. leaf

2.4.2 Traditional uses of Calamus tenuis Roxb.

Calamus tenuis Roxb. is used for treating fever, piles, dyspepsia, biliousness, wounds, bacterial infections [Khare, 2007]. Tag *et al.* (2012) reported that it has anti-diabetic potential. Fruits of *Calamus tenuis* Roxb. are known to have antioxidant and cytotoxic potentials [Ahmed *et al.*, 2014]; analgesic and CNS depressant activities [Hossain, 2013] whereas; its shoots are consumed as vegetables and its occasional consumption is traditionally known to have therapeutic potential against stomach disorder and intestinal worms [Saikia and Khan, 2011].

2.5 Preparation of the plant material and isolation of the active compounds

After choosing the plant, the next step is its collection, botanical identification and stabilization process. It is important that plant recollection involves a professional botanist who is able to correctly identify the species and prepare part of the material for herbarium preservation in order to have a reference material (voucher specimen). Preferably, the place and date of recollection are recorded and the information retained for further collection, if necessary. Stabilization is usually by drying the material at ambient temperature in a shady place, but can also be carried out in an oven with controlled airflow and temperature. When the stability of the plant constituents is unknown or if they are known to be unstable, the fresh plant should undergo a stabilization process consisting of freezing, lyophilisation, use of alcohol vapour, etc [Williamson *et al.,* 1996]. The dried or stabilized plant material should then be powdered and subjected to a suitable extraction process. When the chemical nature of the compounds involved is known (reconfirmation by chemotaxonomic information and databank consultation are crucial), extraction methods should be directed to obtaining these compounds in high yield and purity as possible. When the chemical composition is unknown, the extraction procedure can be based on how the

plant is used in folk medicine, or several extractions with solvents of increasing polarity can be performed [Williamson *et al.,* 1996].

To obtain isolated active compounds, the plant extracts are first qualitatively analyzed by thin layer chromatography (TLC) and/or other chromatographic methods and screened to determine the biological activity or to obtain a general evaluation of biological activities. For purification and isolation, the active plant extracts are sequentially fractionated [Verpoorte, 1989], each fraction and/or pure compound being subjected to bioassay and toxicity evaluation in animals. This strategy is called bioactivity-guided fractionation. Bioassays can be performed using microorganisms, molluscs, insects, cellular systems (enzymes, receptors, etc), cell culture (animal and human), and isolated organs or in vivo (mammals, amphibians, birds, etc) [Hamburger and Hostettman, 1991; Souza-Brito, 1996].

All these methods have advantages and disadvantages and the appropriate method must be carefully selected at each step of any biological study aimed at the development of a drug or the understanding of the biological basis of a particular pathology or even the discovery of the mechanism of action of already known drugs. In general, a plant extract contains low concentrations of active compounds and a large number of promising compounds, requiring the use of sensitive bioassays suitable for the wide chemical variety and small amounts of the tested samples. Tests must be simple, reproducible, fast and cheap [Souza-Brito, 1996; Brito and Nunes, 1997]. Furthermore, new techniques that can fulfill different needs and be adjusted to the classical pharmacological study of natural compounds should be sought. There is also a need for the improvement and establishment of experimental models which are not yet extensively used in the evaluation of natural products.

2.6 Role of plants and its phytoconstituents on diseases

The medicinal plants are used as remedies for various diseases since ancient times. The traditional medicine has served as a source of alternative medicine, new pharmaceuticals, and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds [Mukherjee, 2002]. A significant number of modern pharmaceutical drugs are derived from medicinal plants. The derivatives of medicinal plants are non-narcotic with little or no side effects.

It has been seen that preparation of various drugs from the medicinal plant individually or in combination with other medicines is the key to chronic disease treatment [Mohanta *et al.,* 2003]. Medicinal plants produce bioactive compounds which are used mainly for therapeutic purposes. These compounds from medicinal plants play a crucial role to favour healthy living and protection against various diseases. Phytochemicals referred as "secondary metabolites" are formed during the plants normal metabolic processes, which are of several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [Harborne, 1973; Okwu and Okwu, 2004]. The most commonly encountered secondary metabolites of plants are saponins, tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides and cyanogenic glycosides [Okwu, 2004].

In contrast to synthetic pharmaceuticals based upon single chemicals, many medicinal and aromatic plants exert their beneficial effects through the additive or synergistic action of several compounds acting at single or multiple target sites associated with a

physiological process. Tyler (1999) mentioned that these synergistic pharmacological effects can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body.

Phenols are often considered to be potentially toxic to the growth and development of pathogens [Okwu and Okwu, 2004]. Polyphenols have been reported that it might interfere in several of the steps that lead to the development of malignant tumours which may have role in inactivating carcinogens and inhibiting the expression of mutagens [Urquiaga and Leighton, 2000; Okwu, 2004]. Hypercin, an anthroquinone which is an example of quinine obtained from St. John's wort (*Hypericum perforatum*) has received much attention as an antidepressant, antiviral and also have several antimicrobial properties [Aarts, 1998]. Flavonoids found in varying amounts in foods and medicinal plants have shown to exert potent antioxidant activity against the superoxide radical [Hertog *et al*., 1993], which may be due to its antioxidant activity and subsequent inhibitions of low-density lipoproteins (LDL) oxidation. Flavonoids are potent water-soluble super antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart diseases [Urquiaga and Leighton, 2000]. In terms of anti-cancer activity, they inhibit the initiation, promotion and progression of tumors [Urquiaga and Leighton, 2000; Okwu, 2004]. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions are supported by tannins [Haslam, 1996, Okwu and Okwu, 2004]. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodiac and bactericidal effects [Stray, 1998]. Alkaloids, comprising a large group of nitrogenous compounds are also widely used

as therapeutic agents in the management of cancer [Chabner and Horwitz, 1990; Noble, 1990]. Saponins are a special class of glycosides, which have soapy characteristics [Fluck, 1973] and have shown to be active antifungal agents [Sodipo *et al*., 1991].

During 1950-1970 approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. From 1971 to 1990 new drugs such as ectoposide, eguggulsterone, teniposide, nabilone, plaunotol, zguggulsterone, lectinan, artemisinin and ginkgolides appeared all over the world. 2% of drugs were introduced from 1991 to 1995 including paciltaxel, toptecan, gomishin, irinotecan etc. Plant based drugs provide outstanding contribution to modern therapeutics; for example: serpentine isolated from the root of Indian plant Rauwolfia serpentina in 1953, was a revolutionary event in the treatment of hypertension and lowering of blood pressure. Vinblastine isolated from the Catharanthus rosesus [Farnsworth *et al.*, 1967] is used for the treatment of Hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymophosarcoma, cervical and breast cancer. [Farnsworth and Bingel, 1977]. Phophyllotoxin is a constituent of Phodophyllum emodi currently used against testicular, small cell lung cancer and lymphomas.

2.7 Cancer: A global threat

Cancer is among the leading cause of death globally. The burden of cancer is expected to grow worldwide due to the growth and aging of the population, particularly in less developed countries, in which about 82% of the world's population resides. The adoption of lifestyle behaviors that are known to increase cancer risk,

such as smoking, poor diet, physical inactivity, and reproductive changes (including lower parity and later age at first birth), have further increased the cancer burden in less economically developed countries [Ferlay *et al.,* 2012].

2.7.1 Global scenario of cancer

As per Ferlay *et al.* (2015) report, 14.1 million new cancer cases (Fig-2.7.1.1) and 8.2 million cancer deaths (Fig-2.7.1.2) occurred in 2012 worldwide. The most common cancer sites worldwide are shown gender wise in (Fig-2.7.1.3). Lung cancer remains the most common cancer in the world, both in term of new cases (1.8 million cases, 12.9% of total) and deaths (1.6 million deaths, 19.4%) because of the high case fatality. Breast cancer is the second most common cancer overall (1.7 million cases, 11.9%) but ranks 5th as cause of death because of the relatively favorable prognosis; these are followed, in terms of incidence, by colorectal cancer (1.4 million cases, 694,000 deaths), prostate cancer (1.1 million cases, 307,000 deaths), stomach cancer (951,000 cases, 723,000 deaths) and liver cancer (782,000 cases and 745,000 deaths). These six cancers represent 55% of the global incidence burden in 2012; in more developed regions, just four cancers- female breast, prostate, lung and colorectum (Fig-2.7.1.4) comprise half of the total incidence, whereas lung, female breast, stomach and colorectal cancers combined with liver and cervical cancers explain over half the incidence burden (54%) in less developed regions (Fig-2.7.1.4). The ranking of the 15 most common cancers are shown for men and women (Fig-2.7.1.5), as numbers of new cases and deaths in more and less developed regions of the world. Although lung cancer is the most common cancer worldwide among men, it ranks second in more developed regions (490,000 cases) after prostate cancer (759,000 cases). Cancers of the lung (751,000 cases, 682,000 deaths), liver (462,000 cases, 441,000 deaths) and stomach (456,000 cases, 362,000 deaths) predominate among

males in less developed regions, representing 40% of the new cancer cases and 48% of the total cancer deaths. In women, breast cancer is the most common cancer diagnosed in more and less developed regions, with more cases occurring in less developed (883,000 cases) than more developed regions (794,000 cases). Cervical cancer, the second most common cancer in less developed regions (445,000 cases), ranks 11th in more developed regions (83,000 cases). Lung cancer is the leading cause of cancer death among women in more developed regions (210,000 deaths) followed by breast cancer (198,000 deaths) which ranks as the most frequent in women in less developed regions (324,000 deaths) followed by cancers of the lung (281,000 deaths) and cervix (230,000 deaths). Fig-2.7.1.6 shows the distribution of the global cancer cases and deaths (all types of cancer, both sexes combined) by world region. Most cases (4.1 million, 29.4% of the total) and deaths (2.75 million, 33.6%) occurred in Eastern Asia with its vast population (1.6 billion, 22% of the global population in 2012). Northern America ranks second in terms of number of new cases (1.78 million, 12.7%) but third (691,000, 6.4%) in terms of cancer deaths after South-Central Asia (1.0 million deaths, 12.5%). Almost a quarter of the new cases (3.44 million) and one fifth of the deaths (1.75 million) occurred in the four European regions, despite containing 10% of the global population.

Fig-2.7.1.1: Worldwide cancer incidence

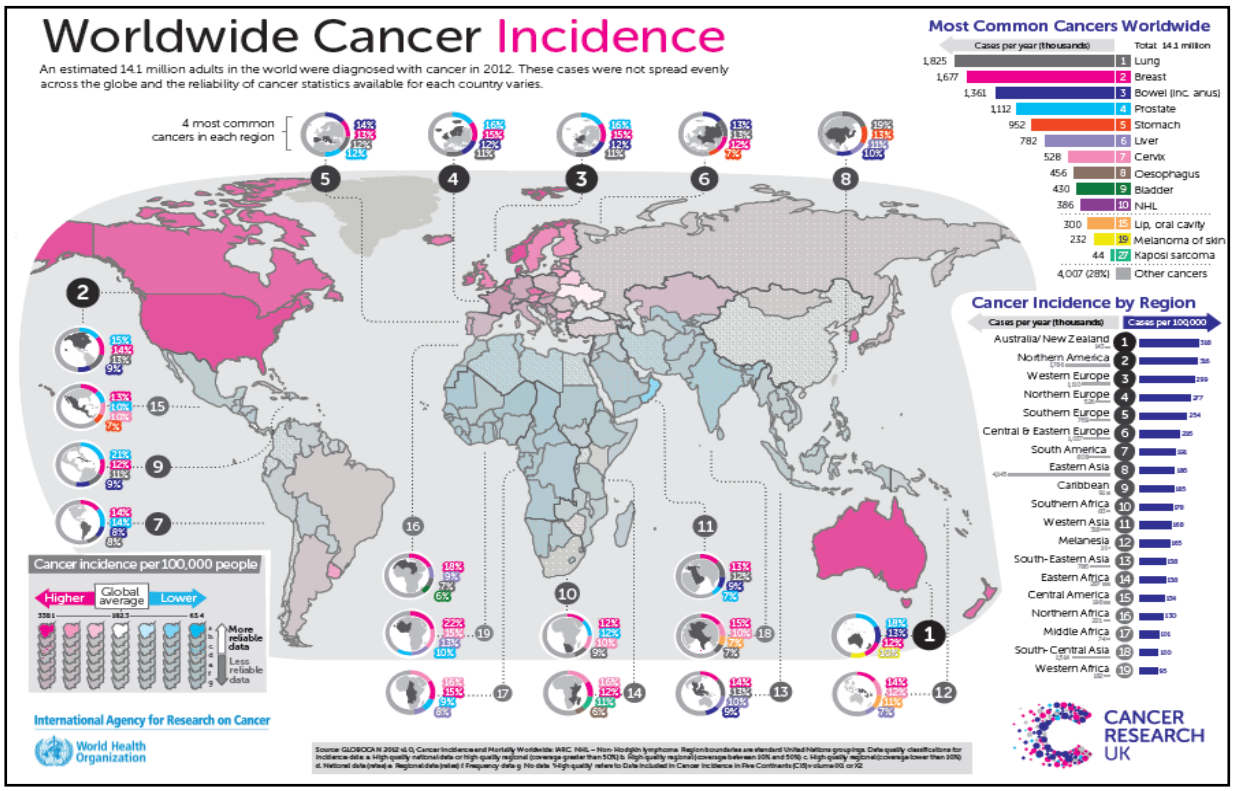


Fig-2.7.1.2: Worldwide cancer mortality

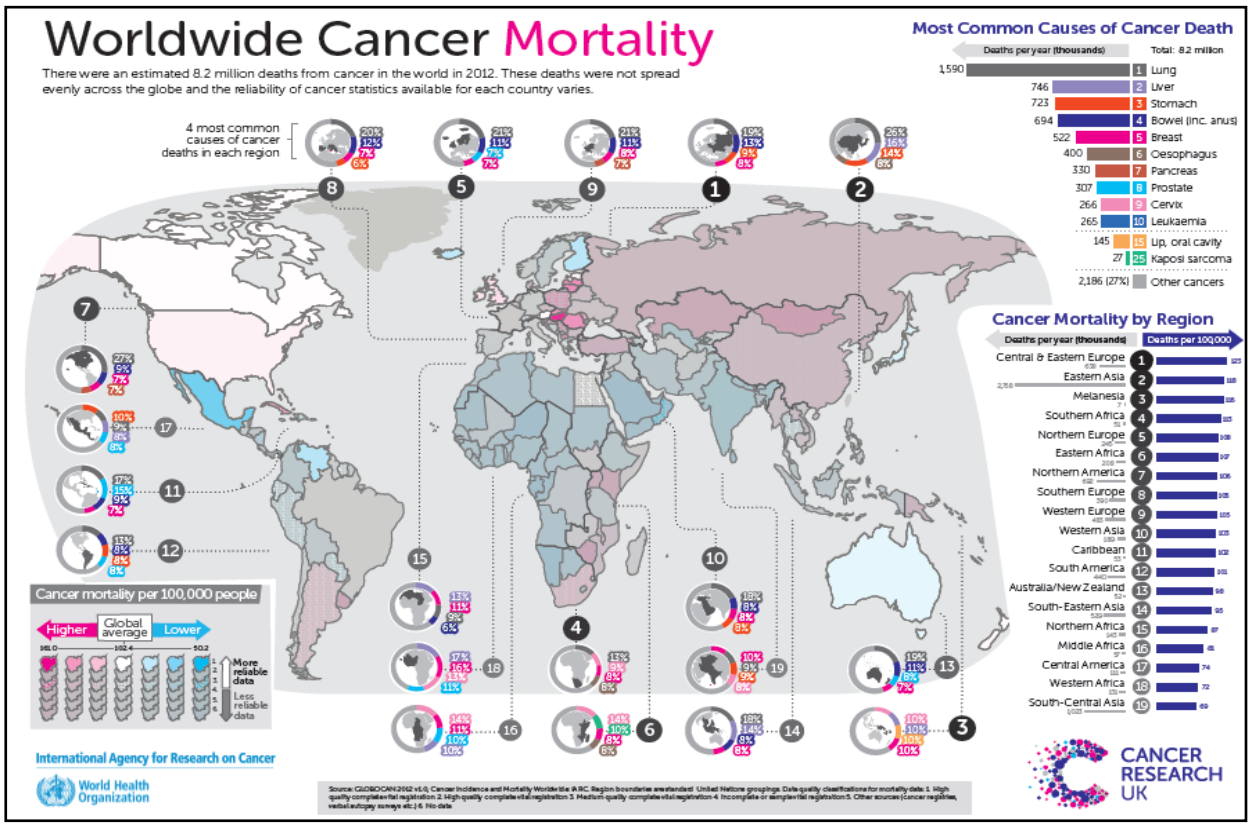


Fig-2.7.1.3: Worldwide gender wise distribution of most common cancer sites

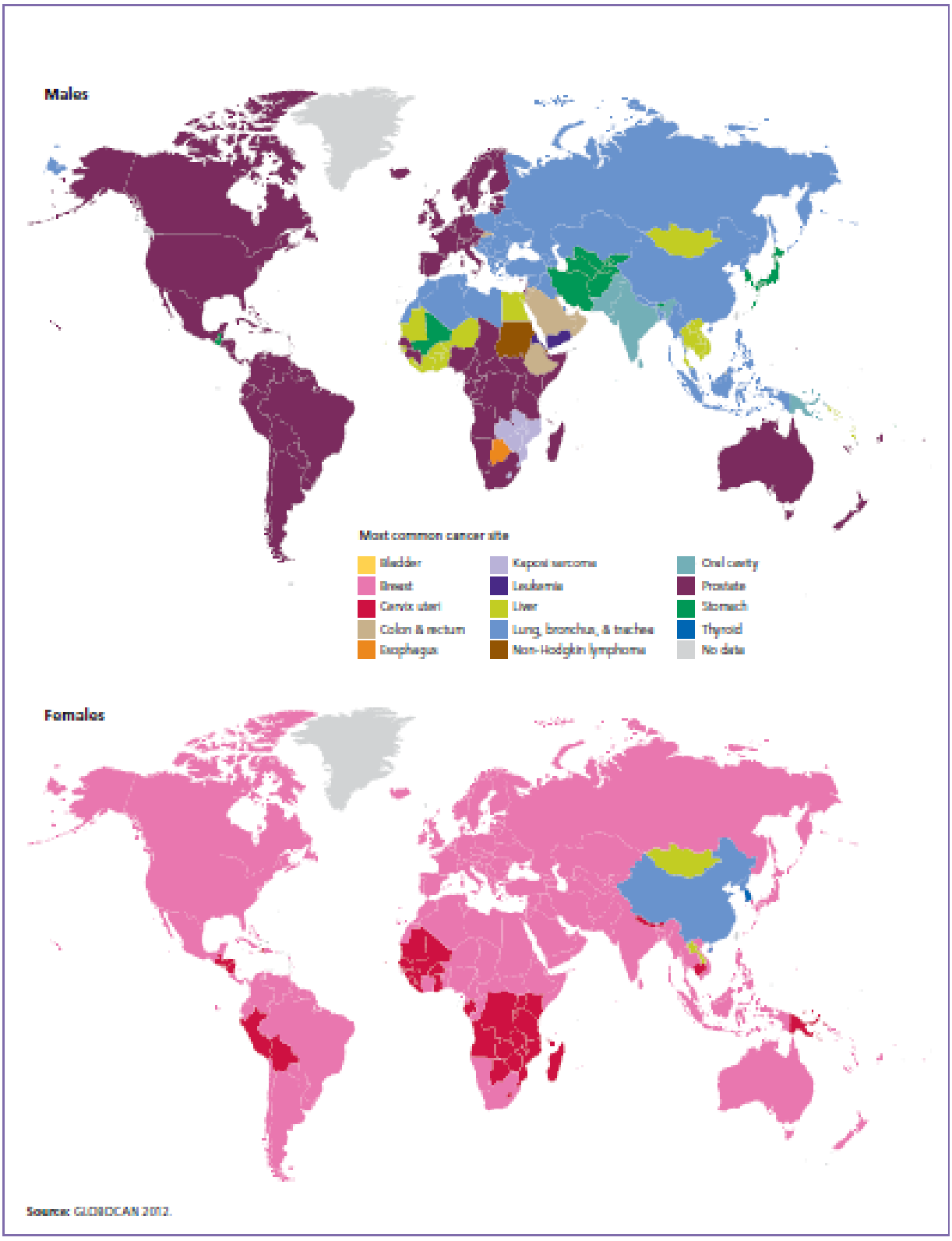
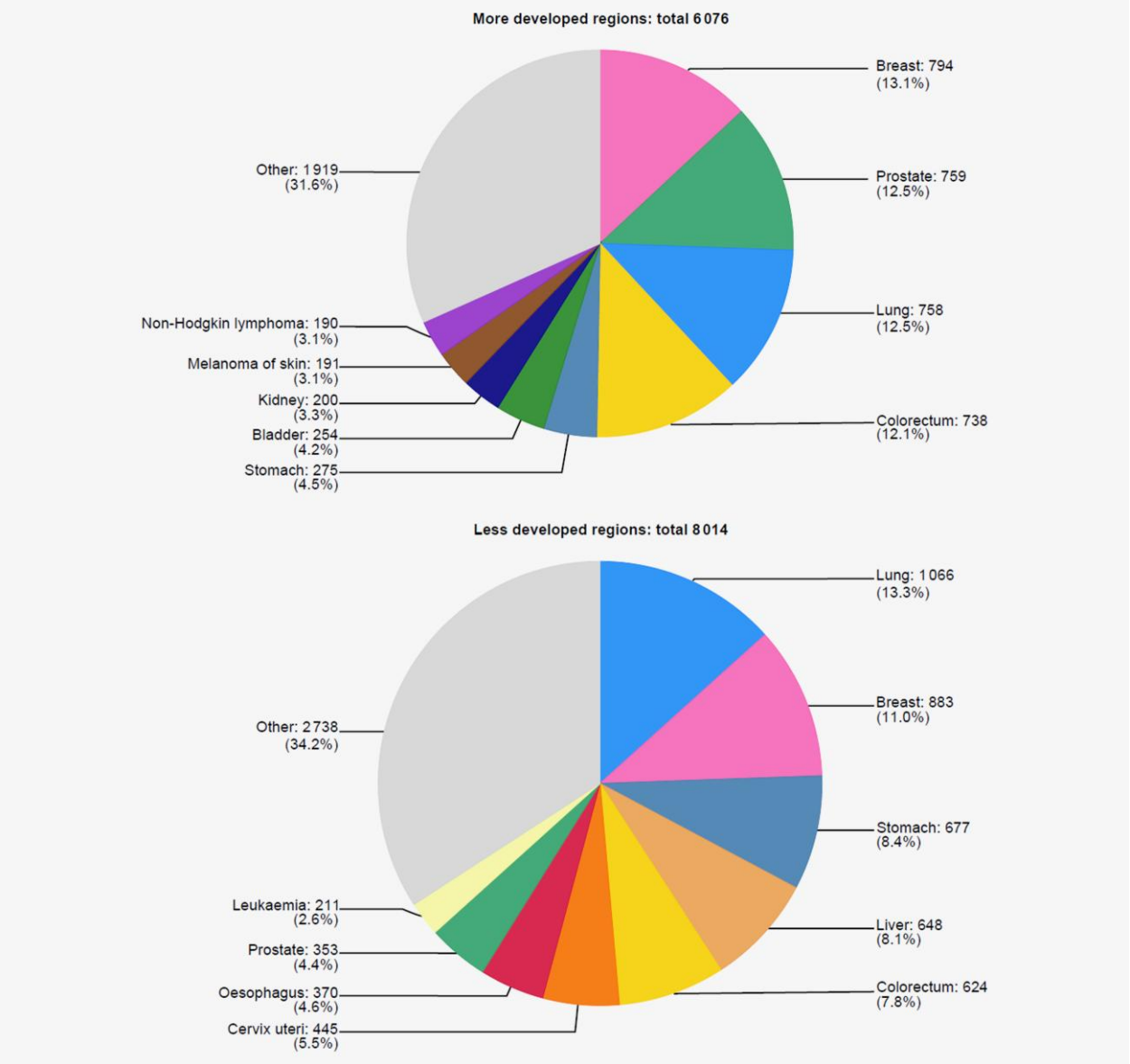
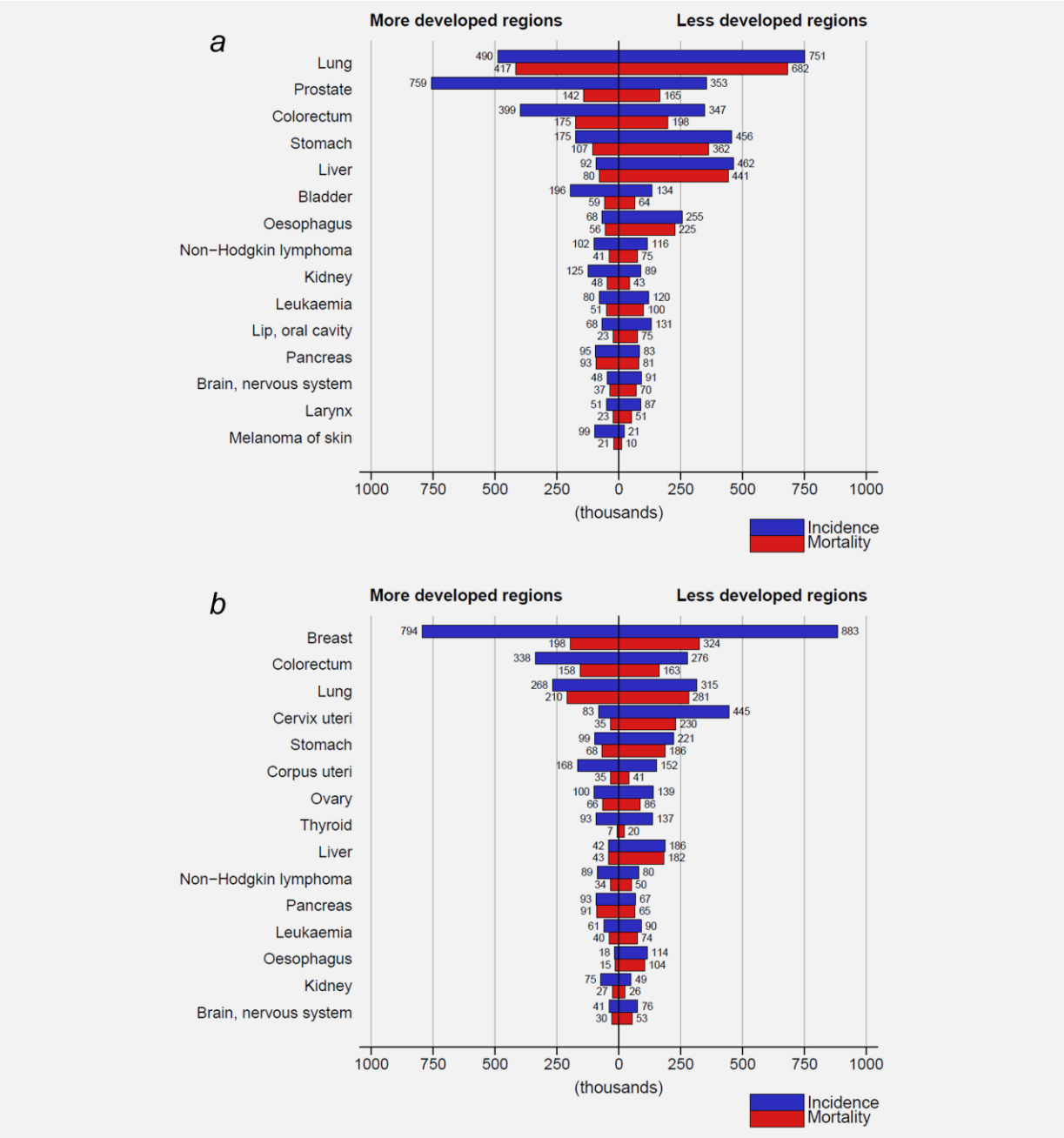


Fig-2.7.1.4: Global estimated numbers of new cases



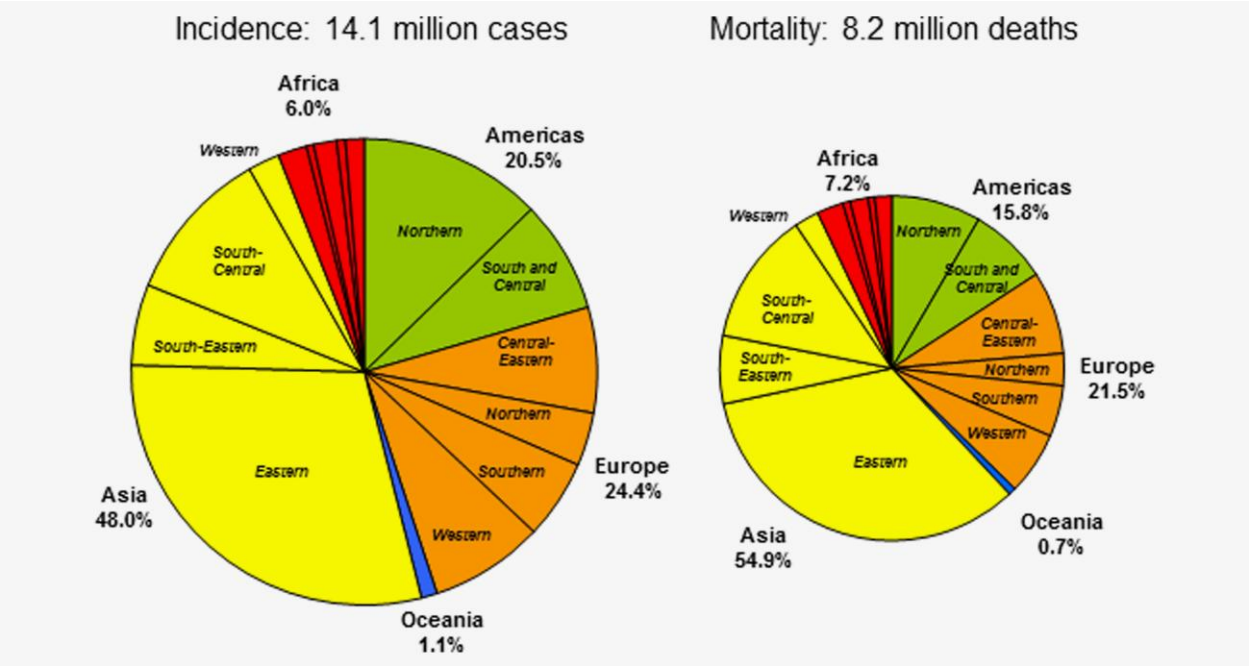
The figure shows the estimated global numbers of new cases (thousands) with proportions for more developed and less developed regions, both sexes combined, 2012. The area of the pie is proportional to the number of new cases.

Fig-2.7.1.5: Estimated numbers (thousands) of new cancer cases (incidence) and death (mortality) in men and women



The figure shows: (a) Estimated numbers (thousands) of new cancer cases (incidence) and deaths (mortality) in men in more developed and less developed regions of the world in 2012. (b) Estimated numbers (thousands) of new cancer cases (incidence) and deaths (mortality) in women in more developed and less developed regions of the world in 2012.

Fig-2.7.1.6: Global new cases and deaths with proportions by world regions, both sexes combined



The figure shows the estimated global numbers of new cases and deaths with proportions by world regions, both sexes combined, 2012. The area of the pie is proportional to the number of new cases or deaths.

2.7.2 Most common cancer type prevalent in men and women

The most common type of cancer found in men and women are lung and breast cancer respectively.

Lung cancer: Lung cancer emerged as the most common cancer worldwide several decades ago: with 660,000 new cases estimated in 1980, it had risen to equal stomach cancer. From the estimated 1.8 million new cases in 2012 (12.9% of the total), 58% occurred in less developed regions. It is the most common cancer in men worldwide (1.2 million, 16.7% of the total), with the highest rates in Central and Eastern Europe (53.5 per 100,000) and Eastern Asia (50.4 per 100,000). Incidence rates are very low in Middle and Western Africa (2.0 and 1.7 per 100,000, respectively). In women, the incidence rates are generally lower and the geographical pattern somewhat different,

reflecting in part variations in the uptake and consumption of tobacco [Ferlay *et al.,* 2015].

Breast cancer: Breast cancer is the second most common cancer in the world and, by far the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). Incidence rates vary nearly fourfold across the world regions, with rates ranging from 27 per 100,000 in Middle Africa and Eastern Asia to 96 in Western Europe. Breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths) and while it is the most frequent cause of cancer death in women in less developed regions (324,000 deaths, 14.3% of total), it is now the second cause of cancer death in more developed regions (198,000 deaths, 15.4%) after lung cancer [Ferlay *et al.,* 2015].

2.8 Mode of action of phytoconstituents on cancer

Once the critical genetic damage has occurred, the next stage in the development of neoplasia are the acquisition of further mutations and epigenetic marks that cause progressively abnormal gene expression. This progression is the promotion stage of cancer development, characterized by poorly-regulated cell proliferation and differentiation, and a reduced tendency for damaged cells to undergo apoptosis [Johnson, 2007].

The emerging evidence for a variety of potentially important anti-carcinogenic mechanisms has stimulated interest in the broad concept of chemoprevention, focused attention on particular fruits, vegetables or plant rich in the most active compounds and encouraged a more mechanistic approach to epidemiology [Johnson, 2007].

2.8.1 Role of phytoconstituents in blocking tumour initiation

Studies of the anti-carcinogenic effects of a variety of plants, plant constituents and synthetic chemopreventive agents led Wattenberg (1985) to define food-borne anticarcinogens generally as either blocking agents, which act immediately before or during the initiation of carcinogenesis by chemical carcinogens, or suppressing agents, which act after initiation during the prolonged stages of promotion and progression. This framework has been valuable for the development of research on the many different types of secondary plant metabolites that show biological activity in mammals [Johnson *et al.,* 1994], and it continues to provide a valuable approach to the classification of these compounds and their effects. A scheme for the interactions between blocking agents, suppressing agents and the generalized sequence for the onset of neoplasia is shown in Figure-2.8.1.

2.8.2 Role of phytoconstituents in modulation of carcinogen metabolism

Blocking agents prevent genotoxic a carcinogen from forming adducts with DNA, either by inhibiting their activation from procarcinogens or by enhancing their detoxification and excretion. These actions are thought to be achieved primarily through the modulation of phase I and phase II metabolic enzymes in the gut, liver and other tissues exposed to the environment. Phase I metabolism involves the oxidation, reduction and hydrolysis of xenobiotics, including drugs, toxins and carcinogens, principally by the cytochrome P450 enzymes [Nelson *et al.,* 1993]. The products of phase I metabolism are often highly-reactive genotoxic intermediates that form substrates for phase II enzymes such as glutathione S-transferase (GST), NAD:quinone reductase and γ-glutamylcysteine synthetase, which then catalyse the formation of less-reactive water-soluble conjugates. These conjugates are then readily excreted via the kidneys or in bile. Certain anti-carcinogenic phytochemicals induce

transcriptional activation of phase I and II enzymes, via the xenobiotic response element and the antioxidant response element respectively. The monofunctional inducers are those that interact primarily with the antioxidant response element, and hence selectively induce phase II enzymes, without simultaneously inducing activation of carcinogens via increased phase I activity [Prochaska and Talalay, 1988]. The mechanism of induction is thought to be triggered by cellular stress, which leads to activation of intracellular protein kinases favouring the translocation of the transcription factor NF-E2 p45-related factor-2 to the nucleus, where it binds to the antioxidant response element and up regulates transcription [Itoh *et al.,* 1997]. Recent findings have shown that a second transcription factor, the aryl hydrocarbon receptor, interacts directly with the NF-E2 p45-related factor-2 promoter, and that this functional link contributes to the coupling of phase I and II enzymes as an integrated system [Miao *et al.,* 2005].

Several groups of phytochemicals have now been identified as potent inducers of phase II enzymes; two of the most actively investigated are the flavanols, including epigallocatechin gallate, which is the principal biologically-active component of green tea [Chou *et al.,* 2000], and the glucosinolate breakdown products, derived from cruciferous vegetables and herbs. Glucosinolates, of which >100 have been identified, are water-soluble glycosides containing a common S moiety and a variable side chain. They are found in cruciferous plants, a large and diverse group that contains mustard (Brassica juncea), rocket (Eruca sativa), radishes (Raphanus sativus) and the brassica vegetables [Mithen *et al.,* 2000]. Intact glucosinolates are biologically inactive and remain sequestered within cells throughout the plant. However, when the raw tissues are mechanically disrupted, the glucosinolates come into contact with the endogenous enzyme myrosinase and undergo hydrolysis, yielding an unstable intermediate that

can give rise to a variety of active products [Mithen *et al.,* 2000]. Depending on the structure of the parent glucosinolate and the ambient conditions, these active products include isothiocyanates (ITC), nitriles, cyanoepithioalkanes and thiocyanates [Lambrix *et al.,* 2001]. In addition, the indolyl glucosinolates give rise to the indole-3-carbinols, which also induce detoxifying enzymes and act as anti-carcinogens in animal models [McDanell *et al.,* 1988]. Glucosinolate breakdown products can be formed in food processing, during digestion of uncooked vegetables in the upper gastrointestinal tract [Rouzaud *et al.,* 2004] and by exposure to bacterial myrosinase in the colon [Rouzaud *et al.,* 2003]. The ITC sulforaphane, derived from broccoli (Brassica oleracea), acts via the NF-E2 p45-related factor-2 pathway [McWalter *et al.,* 2004] and is amongst the most active food-borne monofunctional inducers identified [Talalay and Fahey, 2001].

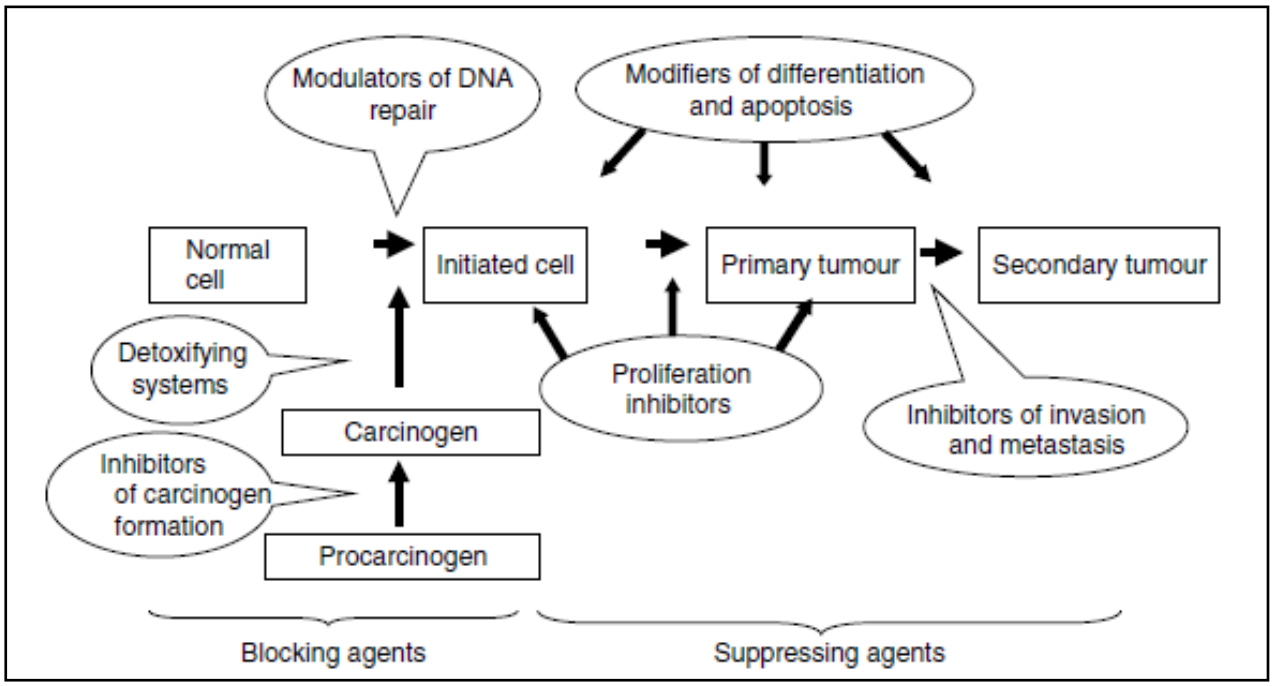
A considerable amount of work has also been done to explore the importance of these effects at the whole organism level by studying carcinogen metabolism in animal models and in human subjects exposed to carcinogens from cigarette smoke. Both indole-3-carbinol and phenethyl ITC have been shown to modify the metabolism of the tobacco smoke carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rodent models and inhibits the development of lung tumours, although their mechanisms of action differ. Indole-3-carbinol induces hepatic a-hydroxylation of NNK, thus reducing the delivery of NNK to the lung [Morse *et al.,* 1990], whereas phenethyl ITC inhibits a-hydroxylation, and hence adduct formation in lung tissue [Hecht *et al.,* 1996]. The shunting of NNK metabolism away from the lung leads to increased hepatic metabolism and higher urinary excretion of NNK metabolites. Increased urinary excretion of two such metabolites, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and [4-(methylnitrosamino)-1-(3-pyridyl) but-1-yl]-β-ω-D-

glucosiduronic acid in human smokers consuming approximately 170 g watercress (Rorippa nasturtium-aquaticum)/day for 3 days suggests that a similar mechanism occurs in human subjects [Hecht *et al.,* 1995]. Hecht and co-workers [Hecht *et al.,* 2004] conducted a study of NNK metabolites in Singapore Chinese smokers and have reported an inverse correlation between cruciferous vegetable consumption and excretion of NNK metabolites that appears consistent with the known metabolic effects of indole-3-carbinol. Overall, the experimental and epidemiological evidence supports the conclusion that glucosinolate breakdown products modulate phase II metabolism in man, although the contribution of this mechanism to the putative anticarcinogenic effects of cruciferous vegetable consumption remains to be fully established.

2.8.3 Role of phytoconstituents in suppressing tumour progression

The cancer phenotype emerges at one or more discrete sites. There are many stages at which this complex process might, in principle, be slowed or interrupted, and a correspondingly large number of plausible mechanisms for tumour suppression. Chemoprevention, the use of drugs or natural compounds at pharmacological levels to inhibit the development of cancer, is currently attracting a great deal of interest, particularly in the USA. However, many of those mechanisms may be triggered by phytochemicals occurring in substantial quantities in human diets.

Fig-2.8.1: A general scheme for interactions occurring between blocking agents and suppressing agents and the various stages in development of neoplasia.



The figure shows the interactions occurring between blocking agents and suppressing agents and the various stages in the sequence of events associated with the stepwise development of neoplasia. Blocking agents act immediately before or during the initiation of carcinogenesis by chemical carcinogens, and suppressing agents act after initiation, during the prolonged stages of promotion and progression (Adapted from Johnson *et al.*, 1994).

2.9 Cytotoxicity potential of various plants and its constituents on human carcinoma and normal cells.

Cytotoxicity tests using specialized cells have proved most useful to clarify the mechanisms of toxic action on the target tissue. These tests have also provided useful insight into the pathogeneses of some human diseases [Klaassen and Stacey, 1982; Auricchio *et al.*, 1985]. Altered cell growth is the indicator of toxicity. The effect of chemicals on the capability of cells to replicate is used as an index of toxicity; the concentration of the substances at which 50 percent of the cells do not multiply is called the median inhibitory dose (ID_{50}). A more specific measure of replication is plating efficiency, the ability of cells (100-200 per dish, 60 mm diameter) to form

colonies after 10-15 days of culture in the presence of a toxic agent gives more complete information, indicating both cell survival and ability to reproduce [Nardone, 1977]. Cell reproduction can be measured by several parameters including cell count, DNA content, protein content, or enzyme activity (e.g. ornithine-decarboxylase) [Costa, 1979]. Another crude index of toxicity is cell viability measured by using vital dyes such as trypan blue which enters dead cells only or neutral red that is actively taken up by living cells; the latter is commonly used in biomaterial testing by the agar overlay method [Guess *et al.,* 1965]. A count of dead and vital cells in comparison with the control provides an index of lethality of the test compound [Holden *et al.,* 1973].

Jayakumar *et al.* (2012) reported that ethyl acetate extract of *Punica granatum* rind (PGR) significantly inhibited the growth of A549 cells as compared to other extracts.

Sundara *et al.* (2013) investigated anticancer activity of various extracts of leaves of *Cadaba fruticosa* in human lung cancerous cell line A549 and 5-fluorouracil and found that leaf ethyl acetate extract was more cytotoxic with a median inhibitory concentration (IC_{50}) of 56.23 µg/ml as compared to Ethanol extract (72.44 µg/ml) and aqueous extract (81.28 µg/ml).

Lee *et al.* (2011) found that *Phyllanthus* plant extracts has inhibitory activity on MCF-7 (breast carcinoma) and A549 (lung carcinoma) cells growth with IC_{50} values ranging from 50-180 mg/ml and 65-470 mg/ml for methanolic and aqueous extracts respectively. In comparison, the extracts had lower toxicity on normal cells with the cell viability percentage remaining above 50% when treated up to 1000 mg/ml for both extracts.

In a study performed by Hoshyar *et al.* (2013), crocin obtained from *Crocus sativus L.* (saffron) plant revealed a dose and time dependent cytotoxic effect against gastric adenocarcinoma (AGS) cell line.

Patel *et al.* (2011) studied cytotoxic activity of methanolic extracts of *Artocarpus heterophyllus* plant by various *in vitro* cytotoxic assays like MTT (3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl-tetrazolium bromide) and SRB (Sulforhodamine B) against different cell lines like HEK293, A549, HeLa and MCF-7. The IC_{50} values of methanolic extract of the plant were found to be 35.26 µg/ml and 35.27 µg/ml against A549 cell line by MTT and SRB assay methods respectively whereas this extract was found to be non toxic to normal cells (HEK293). The methanolic extract had no activity against Hela and MCF-7 cell line.

Silver nanoparticles (AgNPs) synthesized from leaf extract of *Artemisia princeps* induced mitochondrial-mediated apoptosis in A549 cells; conversely, AgNPs had no significant effects on L132 cells. The results from the study suggested that AgNPs cause cell-specific apoptosis in mammalian cells and it also has effects such as anti-bacterial and anti-biofilm activity against *Helicobacter pylori* and *Helicobacter felis* and also cytotoxic effects against human cancer cells [Sangiliyandi *et al.,* 2015].

In a cytotoxicity study conducted on ethanolic extracts of Jordanian medicinal plants, *Curcuma longa* and *Zingiber officinale* showed cytotoxicity potential against A549 lung cancer cells, MCF-7 female breast carcinoma and HT 29 colon adenocarcinoma cell lines [Alkofahi *et al.,* 1997].

Evaluation of cytotoxic potential of Indonesian medicinal plants in cultured human cancer cells carried out by Gowooni *et al.* (2002) showed moderate toxicity of *Zingiber casumunar* to A549 cancer cells.

An isolate (7, 8-dihydroxy flavanone) from the seeds of *Alpinia kastumudai* was found to have *in vitro* cytotoxic activity against A549 (human lung cancer) and K5 (human leukemia) cells [Ryeong *et al.,* 2003].

According to Zaeoung *et al.* (2005) the methanolic extract of *Curnrma longa* has strong cytotoxicity activity against MCF-7 and LS-174 T cell lines, whereas the water extracts of this plants exhibited comparatively less cytotoxic activity.

Lee *et al.* (2005) reviewed and reported the cytotoxic activity of extracts of *Alpinia galanga, A. officinamm, Cayratia japonica, Physalis minima* and *Tabernaemontana divaricola* against human cancer cell lines, *viz.,* CoRL-23 lung cancer and MCF-7 breast cancer cell lines.

In an anticancer study of three solvent extracts of leaves of *Albizia amara* on human breast cancer cells (MCF-7) was found that the ethyl acetate extract had effective cytotoxic action with median inhibitory concentration (IC_{50}) of 36.31µg/ml followed by ethanol extract with 57.54 µg/ml and aqueous extract with 83.18 µg/ml. The ethyl acetate extracts treated cells showed significant signs of apoptosis such as cell shrinkage, membrane blebbing and nuclei fragmentation [Gopinath *et al.,* 2013].

Jannathul *et al.* (2015) reported that biosynthesized silver nanoparticles studied by MTT assay against breast cancer cells (MCF-7 cell line) showed significant cytotoxic activity with IC_{50} value 3.04 ug/ml compared to that of standard cisplatin drug.

Yadav *et al.* (2010) examined 18 second generation curcumin analogues. Of these two compounds showed potent cytotoxicity: 3,5-bis (pyridine-4-yl)-1-methylpiperidin-4-one (RL66) and 3,5-bis (3,4,5-trimethoxybenzylidene)-1-methylpiperidin-4-one

(RL71). Both had IC_{50} values below 1 mM in oestrogen receptor negative cell lines (MDA-MB-231 and MDA-MB-468).

Graidist *et al.* (2015) conducted a cytotoxicity study on crude extracts of *Piper cubeba* against normal fibroblast (L929), normal breast (MCF-12A) and breast cancer (MCF-7, MDA-MB-468 and MDA-MB-231) cell lines and found that the methanolic crude extract of the plant had higher cytotoxic activity against MDA-MB-468 and MCF-7 than a dichloromethane crude extract.

Galvez *et al.* (2003) reported the cytotoxic activity of methanolic extracts of *Plantago coronopus* and *Plantago bellardii* on MCF-7 and UACC-62 cell lines at 32 ug/ml and 34 ug/ml, respectively.

The extracts of *Mangifera indica L.* kernel was reported to have cytotoxicity effect on MDA-MB-231 and MCF-7 breast cancer cells with IC_{50} values at 30 ug/ml and 15 μg/ml respectively, however with low toxicity to normal breast (MCF-10A) cells [Abdullah *et al.,* 2014].

Methanol extract from the leaves of *Melastoma malabathricum* showed significant anticancer activity against MCF-7 cell lines with the IC_{50} value of 7.14 ug/ml while methanol and chloroform extracts from the flowers exhibited a moderate activity towards MCF-7 cell lines with the IC_{50} value of 33.63 ug/ml and 45.76 ug/ml respectively after 72 hours of treatment [Roslen *et al.,* 2014].

The ethanolic extract of *Elephantopus scaber* showed cytotoxic effect towards breast cancer MCF-7 cells with an IC_{50} value of 15 μg/ml. In comparison to the untreated control, the extract triggered cell death with increased phosphatidylserine

externalization, DNA breaks and significant morphological apoptotic characteristics in the MCF-cells [Ho *et al.,* 2011].

Fatope *et al.* (1996) reported that the carotenoid constituent fraction of extract from different parts of *A. senegalensis* plant demonstrated selective and significant cytotoxicity against MCF-7 breast cancer cells (ED_{50} 1.0 μg/ml) and significant lethality in the brine shrimp lethality test (BST) (LC_{50} < 1.0 μg/ml).

Ashidi *et al.* (2010) demonstrated strong cytotoxicity of the methanolic leaf extract of *C. cajan* on human breast (MCF-7), lung (COR-L23) and melanoma (C32) cancer cells (LC_{50} value 0.7-14.7μM) using sulforhodamine B (SRB) cytotoxicity assay. However, the activity was not specific since cell death of the normal skin epithelial cells (SVK14). Further fractionation of the methanol extract yielded six bioactive compounds (hexadecanoic acid methyl ester, α-amyrin, β-sitosterol, pinostrobin, longistylin A and longistylin C) believed to be major contributors of the observed cytotoxicity. An isoflavanone, phytoalexin and cajanol (5-hydroxy-3-(4-hydroxy-2-methoxyphenyl)-7-methoxychroman-4-one) was considered as most active principle isolate from the roots of *C. cajan*. Cajanol has been reported to induce apoptosis of MCF-7 cells in a time and concentration dependent manner by Bcl-2/Bax-mediated mitochondrial apoptotic pathway [Luo *et al.,* 2010b].

Essential oil of *Cymbopogon citratus* (lemongrass) and *Cymbopogon nardus* (citronella), as well as the monoterpenic aldehyde citronellal and its chemically modified product C37A (*N*-citronellylamine) were evaluated for their cytotoxic potential. Two cell lines were used, a breast cancer cell line (MCF-7 - ATCC HTB-22) and a non-tumorigenic cell line (Vero - ATCC CRL-1586). Using crystal violet assay, it was observed that all tested compounds demonstrated cytotoxicity for both

cell lines, however with different intensities. Lemongrass oil was cytotoxic in similar way for both cell lines, while the others presented higher cytotoxic response to the tumor cell MCF-7, being citronellal a high selective product. The chemical modification of citronellal that originated the C37A, promoted higher cytotoxic effects but no selectivity [Stone *et al.*, 2013].

Samarakoon *et al.* (2016) tested four different solvent (hexane, chloroform, ethyl acetate and methanol) leaf extracts of Mangrove plant *Phoenix paludosa* Roxb for cytotoxicity against four cancer cells namely MCF-7 (oestrogen positive breast cancer), MDA-MB-231 (triple negative breast cancer), SK-BR-3 (breast adenocarcinoma) and ACHN (renal adenocarcinoma) as well as two normal cell lines-HEK-293 (embryonic kidney cells) and MCF-10A (normal mammary epithelial cells) and found that, of the four extracts, methanol extract showed the strongest significant ($p < 0.05$) cytotoxicity to all four cancer cell lines at 24 and 48 h of incubation followed by the chloroform extract (IC_{50} of methanol extract (24 and 48 h): 36.71 ± 8.72 and 33.19 ± 5.53 μg/ml (MCF-7), 159.7 ± 32.09 and 141.9 ± 26.2 μg/ml (MDA-MB-231), 103.3 ± 18.9 and 75.39 ± 19.39 μg/ml (SKBR-3), 57.21 ± 3.72 and 43.16 ± 10.25 μg/ml (MCF-10A), 37.48 ± 5.75 and 26.99 ± 1.85 (ACHN) and 66.83 ± 14.26 and 60.34 ± 10.66 μg/ml (HEK-293). Furthermore, the methanol extract was least cytotoxic to normal cell lines.

Ali (2014) investigated *in vitro* cytotoxicity activities of 14 wild angiosperms against human breast adenocarcinoma (MCF-7) cell lines by MTT and found that the ethanolic extract of *Lavandula dentata* (Lamiaceae) exhibited promising cytotoxic activity with an IC_{50} value of 39 μg/ml.

Srisawat *et al.* (2013), reported that the acetone and methanol extracts of the fruit cotyledons of *Vatica diospyroides* Symington Type LS extracts were highly cytotoxic against MDA-MB-468 cell line (IC_{50} = 3.1 μg/ml).

Mahani *et al.* (2014), investigated the selective cytotoxic properties of *Thymus caramanicus* extracts on MCF-7 human breast cancer cells and found that the concomitant treatment of cells with extract and anticancer drug produced a significant cytotoxic effect as compared to extract or drugs alone.

Gingko biloba, Ipomoea carnea and *Lonchocarpus speciosus* extracts exhibited markedly high selectivity index (SI) values with colon cancer [Seham *et al.,* 2014].

Cytotoxicity investigation of *Hibiscus sabdariffa* aqueous extract (HSE) on human breast adenocarcinoma cell line (MCF-7) and fetal foreskin fibroblast (HFFF) showed that the viability of MCF-7 cells was less than 50% at the concentration of 0.5 mg/ml following 72 hours of incubation. The extract was non cytotoxic against normal HFFF cells in all tested concentrations. Also, HSE was found to induce apoptosis only in MCF-7 cells [Khagani *et al.,* 2011].

The methanolic extract of two hundred wild and cultivated plants growing in Egypt were screened by *in vitro* (MTT) for cytotoxicity against four human cancer cell lines namely breast (MCF7), colon (HCT116), hepatocellular (HepG2) and lung (A549). Remarkable cytotoxic activities were obtained with fifteen tested extracts (at 100ppm) against all the four tumor cell lines. Four plants namely *Dovyalis caffra, Gingko biloba, Ipomoea carnea* and *Lonchocarpus speciosus* possessed high cytotoxicity against the majority of the tested tumor cell lines. The selectivity index (SI) was also estimated for these four plant extracts using human normal skin cell line (BJ-1). On the bases of SI values, the branch extract of *Dovyalis caffra* showed relatively high

selectivity to the lung tumor cell line. The methanol extracts of leaves of *Gingko biloba and Ipomoea carnea* and the bark extract of *Lonchocarpus speciosus* exhibited markedly high SI values with colon cancer [Seham *et al.,* 2014].

Thus the above discussed review depicts that ensuring the social value of research includes devising and implementing sound science. Although international collaborative research on herbal medicine is no exception, discussing scientific validity as an ethical requirement raises some specific challenges, including the validity of research strategies, establishing inclusion and exclusion criteria, using appropriate outcome measures, and determining appropriate study designs.