Herbal Medicine

Adam and Eve lived in heaven were neither familiar about disease nor suffering; but when they were expelled and then discovered misery and disease.

Necessity is mother of invention, therefore, since ancient time man has searched for remedies to combat against several diseases. Historically as we know that, man has explored the nature for two major needs- food for survival and herbs for relieving of pain and diseases. Comprehensive treatments used by ancient civilizations were herbs or mixtures of them named as corpus therapeuticum to cure disease. One of these compound was Ebers Papyrus. Egyptian culture used medicinal herbs were explained in the Ebers Papyrus in about 1550 BC back. German Egyptologist Dr. George Ebers purchased the papyrus in Thebes in 1872 and recognized its components and extraordinary values. Indian Ayurveda (‘science and knowledge of life’) was discovered and has been used in India since thousands years back 900 BC (Ebbell, 1937).

Now-a-days, in India, medicinal plants have been used under a medical system Ayurveda since 5000 years. This system includes diet and herbal remedies emphasizing to the body, mind and spirit for disease prevention and treatment (Morgan, 2002).

In world wide, consumers are playing positive intention toward the herbal products, and believe them to be of ‘natural’ rather than ‘synthetic’ origin, and also assume that such products are more likely to be safe than synthetic drugs. They are considering a part of healthy life style and started to avoid to contact with conventional ‘western’ medicine.

It has been reported that herbal drugs and their constituents have advantageous effects on long-term fitness and can be used efficiently to treat human diseases or disorders (Luqman et al., 2014).

Mankind have explored herbal medicines for pain relieving and to cure various diseases since immemorial time. Besides above, use of medicinal plant has
been prevailing since 60,000 years ago, Sumerian clay slab (a type of equipment) was discovered for verification of medicinal plants to synthesis of drugs (Sumner, 2000). Now a days, more than 50% natural drugs are being used for medication, originated in some way from plants (Yarnell and Abascal, 2002; Fabricant and Farnsworth, 2001).

Presently, herbal medicines are often used for health care in both developed and developing countries. Herbal medicines are known as mixtures of chemical products synthesised in plants, and have limited effectiveness due to poor absorption by oral administration (Gautam et al., 2003; Sagar et al., 2005). According to survey of the World Health Organization (WHO), about 80% of the world population are using herbs and other traditional medicines for their primary health care (www.who.int/research/en). As per rule of WHO, three kinds of herbal medicines are established: raw plant material, processed plant material and herbal products (Choudhary and Sekhon, 2011).

It is fact that herbal medicines are natural products and proved to be safe due to less side effect while using against diseases along with dietary supplements for forcefully prevent from diseases. Looking to the demand, now-a-days herbal products are sold as tablets, capsule, powder, tea, extracts and fresh or dried plants (www.morethanvitamins.co.uk/herbal-remedies-c-24.html). Herbals are traditionally known harmless, hence people do not hesitate to consume without any prescription. Although, some may cause health problem and some are non-effective and interactive with other drugs (Choudhary and Sekhon, 2011). In present scenario, assessment of drugs quality and standardization of herb products seems to be necessary to assess the concentration of their active ingredients (Yadav and Dixit, 2008).

**Why medicinal plants**

Many reasons have explained on using of medicinal plants as therapy. It is believed that medicinal plants are more effective than orthodox medicines. Consumers also preferred natural therapies and have more interest in herbal drugs to an extent superiority over synthetic drugs. Bagnis et al. (2004) have explained that some communities of south Africa believe that use of traditional medicinal can clean the negative influences of spirituals.
In addition, people are dissatisfied with uses and results from synthetic drugs and believing that natural drugs could be more effective in curing of several hazardous diseases, where conventional therapies and modern medicines are failed. Therefore, most of the people are paying attention on herbal medicines for treatment of diseases especially in developing countries because non-availability of professional care, inconvenience, costly, and time consuming (Gill et al., 1994).

In rural areas, additional culture factors are very much encouraging for using of plant products and this concepts interplay the role between nature and culture, a “man-earth” relationship (Gester, 1992). With the development of science and technology, the improvement in the quality, efficacy, and safety of herbal medicine is becoming widely responsible for use of medicinal plants. In many cases, patients also believed that their physicians are not able to identify the real problems, hence they are forced to move for herbal remedies as another option (WHO, 2002; 2005).

**Turmeric as Herbal Drug:**

The herbal products are being used since ancient period for various purposes. Natural products are coevolved with earth life, and are older than billions of years. Out of thousands plants, ten produce secondary metabolites against various diseases and infections. Herbal drugs have played significant role in health improvement since both ancient and modern (Butler, 2004; Newman et al., 2007). Presently, the holistic medicines of Indian system are known as Ayurveda mainly plant-based drugs or formulation to cure the many ailments including cancer. Presently drugs as small-molecule level are introduced worldwide, most of them has been traced back to their origins from natural products (Newman et al., 2003).

*Curcuma longa* is Latin name of turmeric which derived from the Arabic plant named as Kurkum. Many different names for turmeric are known in different language in Sanskrit “Haridra” (The yellow one), “Gauri” (the one whose face is light and Shining), “Kanchani” (Golden Goddess), and Aushadhi (“herb”). It is called as “Jiang Huang” in Chinese, and “Haldi” (“Yellow”) in Hindi, the most commonly known in India (Frawley et al., 1993).
The two varieties of turmeric are characterised with hard, rich colored, oval rhizomes, called “Lokahandi Halad” that is used mostly for dying, and other varieties are softer, larger, light colored with long rhizomes used mostly for eating (Nadkarni, 1976).

Turmeric has been assigned as a household plant and is used in different folklore prescription, and over the counter (OTC) drugs since ancient times (Assawapantanakul, 1981; Toniwat, 1978). The rhizome is administered orally for regulation of health. Its choleretic action enhanced bile flow into the intestine and is also a remedy for dyspepsia and diarrhoea (Pongbunroad, 1965). The paste is made by dissolving turmeric in water and applied on the skin to kill bacteria, fungi, and other pathogenic microbes, to heal wounds and reduce itching in skin diseases (Pongbunroad, 1965; Bunyapraphatsara, 1986). It is also cooked with coconut oil, and used as an astringent in wounds (Pongbunroad, 1965; Bunyapraphatsara, 1986). Moreover, turmeric has been used as cosmetics for ladies by applying the mixture of turmeric and honey over the body skin. Water extract of turmeric powder mixed with breast milk can be used for treatment of conjunctivitis in baby (Assawapantanakul, 1990).

For cultivation of turmeric in agricultural field, its rhizomes are propagated. Most favourable season for growing of turmeric is rainy season especially in the months from May to July. In winter its leaves becomes shaded off and rhizomes remain under the soil. In next rainy season, the plant sprouts and grows again. The harvesting period starts from 7 to 9 month after planting the crop mainly from December to February (Prucksunand et al., 2001).

The Ministry of Public Health declares the turmeric as fundamental drug for the primary health care. Turmeric is one of the five medicinal plants used extensively as single drug i.e. not along with other plants components combination for the formulation (Suppasil-Nanakorn, 1993). Turmeric is also known as condiment and as spice and exported approximately 39.8 tons per year. It is used by the traditional medicinal industry (approximately 6.036 tons per year) (Bunyapraphatsara et al., 1981). Our ancestors have believed that human body is consisted of four elements namely, earth, water, air, and fire.
The digestion is a type of fire elements, in which turmeric promotes health by regulating the fire elements (Sethaputra, 1980; Bunyapraphatsara, 1992).

*Curcuma longa* is a tropical plant and it is extensive cultivated in Asia, India, China, and other countries at favourable climates. *Curcuma longa* belongs to *Zingiberaceae* family, characterised as a perennial herb. Dimensionally, it can grow up to 1 m high, and contain oblong, tufted leaves. The yellow color spices are made after boiling, drying and then grinding of rhizomes (Dobelis, 1986; Bharat et al., 2005). Curcumin contribute the yellow color is an active component of turmeric, and it is found from 2 to 8% of the rhizomes (Heath et al., 2004). Curcumin is a hydrophobic curcuminoid and quite soluble in organic solvents such as DMSO, ethanol, methanol, or acetone (Tonnessen and Karlsefn, 1985), hence ethanol extraction method is being used for its extraction from turmeric powder (Dobelis, 1986). *Curcuma longa* is used in Asian cuisine as coloring agent of cheese, butter, yoghurt, and other kinds of food (Arun and Nalini, 2002). In addition, curcumin has a wide spectrum of biological and pharmacological activities. Chemical nomenclature is a bis α, β-unsaturated β-diketone and commonly called as diferuloylmethane, which exhibits tautomerism keto-enol, having keto form in acidic and neutral solutions and stable enol form in alkaline medium (Preetha et al., 2007). The several studies have revealed that curcumin are used as antioxidants and anti-inflammatory (Hsu and Cheng, 2007)). Currently, many studies have also shown that curcumin interferes with the activity of cellular enzymes, and angiogenesis (Chainani-Wu, 2003; Sharma et al., 2005). The first study on curcumin and its dose-limiting toxicity was not understood until 2001. According to earlier reports, the toxic effect of curcumin was not observed upto 8 g curcumin administered per day in human (Cheng et al., 2001).

**Turmeric and Ayurveda**

Turmeric has been used since 5,000 year old as natural healing in India, and for centuries in Ayurveda. Forty six different synonyms of turmeric are known including: “ptta” (yellow), “gauri” (brilliant) and all words indicate “night” (Williamson and Elizabeth, 2002), this brings from a tradition of married women who apply the turmeric on their cheeks in evening in preparation to
visit of Lakshmi (The Goddess of Prosperity) (Majeed et al., 1995). In Ayurveda, turmeric is used as healer medicine taken orally in the form of fresh juice, boiled tea, tinctures, or powder, and typically as creams, lotions, pastes, and ointments (Whole Health MD.com). Ancient Ayurvedic has formulated the turmeric for utilising to care and improve the health, for example the boiled milk with turmeric and sugar for cold remedy and turmeric juice for healing the wounds, bruises, and leech bites. The turmeric smoke was used to relieve scorpion stings within a few minute. Inhaling the burnt turmeric fumes was also useful to release copious amounts of mucous and provide instant relief from congestion. The pinch of turmeric also acted as an insect repellent in the kitchen (Kaushik and Purshotam, 2003). The paste of either turmeric alone or with neem (Azadirachta indica) leaves was used to cure of ringworm, itching, eczema, and any other parasitic skin problems (Nadkarni, 1976). Pastes of turmeric were also used for smallpox, chickenpox, shingles, ulcers, conjunctivitis, skin blemish, malaria, and applied to cut the placenta after the birth of a child (Nadkarni, 1976). About hundreds of molecular constituents are found in turmeric, and each have different biological activities. Among them at least 20 molecules are anti-biotics, 14 cancer preventives, 12 anti-tumor, 12 anti-inflammatory and about 10 different anti-oxidants (Majeed et al., 1995).

One database explained about 326 known biological activities of turmeric (www.umm.edu/altmed/ConsHerbs/Turmericch.html). The rhizomes contain minimum 70% carbohydrates, 7% protein, 4% minerals, and 4% essential oils. In addition, it contains vitamins, other alkaloids, and about 1% resin (Majeed et al., 1995). The active components of turmeric is called as curcumin containing 2-5% in turmeric rhizomes (Sundaram et al., 2005).

**Science and Turmeric**

Scientists are initiating their interest in turmeric by realizing the importance of turmeric in treatment of several modern diseases. Many studies have been conducted on various effect of turmeric with human body and the most attention has been made related to cancer. Turmeric is considered to fight against cancer in three ways: 1) It neutralizes those substances and
circumstances which can cause cancer, 2) it helps directly to maintain the cell integrity if threatened by carcinogens, and 3) often destroy the growing tumor (Kawamori et al., 1999). Many reasons have explained about destroying cancer. One of the main activities of curcumin is to inhibit the enzyme Topoisomerase, which plays essential role in the DNA replication in cancer cells. “Topoisomerase” works inside the nucleus of cell, where it first binds to supercoiled DNA and then catalyzes the passage of one DNA helix through another via a transient double-standard break. This splits the DNA and thus allows fast replication of cell to occur. Inactivation of topoisomerase activity by curcumin stops the replication and reduce the spread of the problem (Kawamori et al., 1999).

Allopathic methods are also being used to treat the cancers, where turmeric has been found more efficient with no side effects in cancer treatment. Recent researches have suggested the use of turmeric with diet can suppress the proliferation of cells (Kawamori et al., 1999). Turmeric also has a unique potential to purify and nourish the blood and skin. External applications of turmeric reduces pain and swelling, and heals wounds rapidly, and cure skin diseases ranging from acne to leprosy (Prashanti, 2003).

Turmeric also protects the liver from toxins and pathogens by destroying toxins and rebuild the liver (Prashanti, 2003). Turmeric stimulate the secretion and movement of bile, and protect the liver from diseases. It has been advised that about 5 g of turmeric with a glass of whey in morning and evening for a month activates the liver function (Nadkarni 1976).

Traditionally, turmeric is being taken orally in the last two weeks of pregnancy in to expedite a easy birth, as well as for protection of the mother and child health. Turmeric acts as analgesic to relief the pain during childbirth (Prashanti, 2003).

Turmeric plays also a vital role in the entire gastro-intestinal systems by increasing intestinal flora and generate healthy digestion (Frawley et al., 1993). It is traditionally used for stomach, poor digestion, dyspepsia, parasites, abdominal cramps, normalize metabolism, protein digestion and breakdown of fats, to increase absorption, and provide support to the stomach for
maintenance the secretion of digestive acids (Frawley et al., 1993). Turmeric is also a carminatives: that helps to decrease gas and distention. According to Ayurveda, plants acting in digestive processes are considered to important herb, because this keeps mental and physical health. As a vulnerary it also helps to nurture and heal mucous membranes; and protect against foods and materials that are corrosive to the stomach and intestines.

Another major use of turmeric has been reported in case of the respiratory system. As an anti-oxidant it protects the lungs from pollution and toxins. It also helps the transportation oxygen from the lungs to the blood. Traditionally, turmeric with ghee has been used to get relief from cough and asthma (Nadkarni, 1976). It also supports the heart in many ways by lowering cholesterol and prevent atherosclerosis (blockage of arteries that can cause heart attack or stroke) (www.umm.edu/altmed/ConsHerbs/Turmericch.html). Experiment with animal showed that turmeric lowers cholesterol levels and inhibits the oxidation of LDL (“bad cholesterol”) responsible for clogging of arteries).

Studies are also underway regarding turmeric use in preventing neurological diseases such as Multiple Sclerosis and Alzheimer’s Disease. It is believed that elder Indian people who eat turmeric regularly are away from these ailments. Although, Alzheimer’s Disease is 4.4 times less among older and adults people in India in comparison to United States (Sundaram et al., 2005).

Overall turmeric can obviously stand the test of time. Due to its great uses in worship revealed and revered by people since centuries, and till today it is one of the most significant players in the prevention of serious diseases. Much more researches are going on to prove scientifically about the knowledge of ancient people of India since centuries and concluded that turmeric is one of the most powerful plants on this planet. It is suggested that if suffering from an acute or chronic disease, or as preventive maintenance, turmeric should be utilized by everyone on regular basis. In the words of David Frawley: “If I had only a single herb to depend upon for all possible health and dietary need, I would without much hesitation choose the Indian spice turmeric” (Prashanti, 2003).
Main constituent of turmeric curcumin was isolated first in 1815 and obtained in crystalline form in 1870 (Daybe, 1870; Aggarwal et al, 2007). It was chemically identified as 1, 6-heptadien-3, 5-dione-1, 7-bis (4-hydroxy-3-methoxyphenyl)-(1E, 6E) or diferuloylmethane. The feruloylmethane of curcumin was synthesised and confirmed by Lampe (Lampe et al., 1913). The melting temperature 183 °C a molecular formula of C_{21}H_{20}O_{6}, and a molecular weight of 368.37 g/mol of curcumin were recorded. Curcumin pH between 2.5-7.0 appears brilliant yellow and red at pH > 7. The stability of curcumin depends on pH conditions, at acidic curcumin exhibits as stable and is degraded into ferulic acid and feruloylmethane at basic condition (Oetari et al., 1996; Wang et al., 1997). The slow degradation of curcumin occurs between pH 1-6 (Wang et al., 1997), therefore normally encountered in stomach. It is used traditionally in China to treat diseases related to abdominal pain (Aggarwal et al., 2004). In ancient Hindu medicine, curcumin was used to treat sprains and swelling (Araujo et al., 2001). Curcumin has been used as good therapeutic agents against anti-inflammatory (Aggarwal et al, 2007) and its several therapeutic action against various diseases have been confirmed by modern researches such as antioxidant (Sreejayan and Rao, 1997), anti-inflammatory (Ammon and Wahl, 1991; Brouet et al., 1995), anticarcinogenesis, hepatoprotective and antimicrobial (Kiso et al., 1983; Rao et al., 1995), thumbosuppresive (Srivastava et al., 1985), cardiovascular (protection against myocardial infarction) (Nirmala and Puvanakrishnan, 1996), hypoglycemic (Srinivasan, 1972), and antiarthritic (protection against rheumatoid arthritis) (Deodhar et al., 1980). The most compelling and continuing traditional therapeutic use of curcumin is its extremely safety profile for health. To date, there is not toxicity associated with use of curcumin observed in animal (Shankar et al., 1980) or human (Lao et al., 2006), and indicated that curcumin is not toxic even at very high doses.

**Interaction of curcumin with various targets**

Curcumin is likely a highly pleiotropic molecule that interacts physically with its numerous targets. Curcumin inhibits the activity of enzymes, growth factor receptors, metals, albumin, and other molecules, as stimulator for progression of diseases. It binds with several proteins such as P-glycoprotein
(Anuchapreeda et al., 2002), multidrug resistance protein 1 and 2 (MRP1 and MRP2) (Wortelboer et al., 2003), glutathione (Wortelboer et al., 2003), protein kinase C, ATPase (Logan-Smith et al., 2001), ErbB2 (Jung et al., 2007), and alpha1-acid glycoprotein (AGP) (Zsila et al., 2004). Curcumin stops aggregation and formation of fibril in vitro as well as in vivo which bind with small β-amyloid species (Yang et al., 2005). Irreversible binding of curcumin to CD13/aminopeptidase N (APN) inhibits the angiogenesis as well as tumour invasion (Shim et al., 2003). Another activity of curcumin also revealed binding with lipoxygenase (Skrzypczak-Jankun et al., 2003) or phosphatidylcholine (PC) micelles (Began et al., 1998) and inhibition of lipoxygenase.

**Curcumin inhibits activation of transcription factors**

Curcumin plays a vital role to inhibit the activation of several transcription factors like nuclear factor-kB (NF-kB), activated protein-1 (AP-1), signal transducer and activator of transcription (STAT) proteins, peroxisomes proliferation-activated receptor-γ (PPAR-γ), and β-catenin (Shishodia et al., 2007), which regulate gene expression contributing in tumorigenesis, inflammation, cell survival, cell proliferation, invasion, and angiogenesis.

**Curcumin down regulates the multiple kinase activity**

A types of tyrosine kinases are activated by mutation that play role in malignant transformation, growth, and metastasis in human cancers. According to many reports, protein kinases participated in growth signaling cascades are novel target site for chemopreventive approaches to cure various human cancers. Likewise, many human cancers overexpress the epidermal growth factor receptor (EGFR) and HER2/neu which trigger the proliferation of cancer cells (Lengyel et al., 2007). On the above circumstances, in vitro cellular experiments with curcumin treatment showed inhibition of EGFR kinase activity and EGF-induced tyrosine phosphorylation of EGFR in A431 cells and reduced Her2/neuprotein in cells. In place of geldanamycine, curcumin can also degrades intracellular HER2 and destroys its tyrosine kinase activity (Tikhomirov and Carpenter, 2003). Moreover, curcumin induces apoptosis in acute T cell leukemias by inhibiting the phosphatidylinositol 3 kinase/AKT
pathway and arresting the G2/M of cell cycle and inducing non apoptotic autophagic cell death in malignant glioma cells by disrupting Akt and Erk signaling pathways (Tikhomirov and Carpenter, 2003).

**Curcumin inhibits expression of growth and metastases promoting genes**

Overexpression of oncogenes stimulates the growth of tumor cells and provide the target for chemopreventive regimens. Various types of cancers such as colon, lung and breast are associated with cyclooxygenase-2 (COX-2). Research in past decades has focused on the development of COX-2 inhibitors (Grosser, 2006) and reported that curcumin inhibits the expression of COX-2 through downregulation of NF-kβ activation essential for COX-2 activation.

**Curcumin inhibits expression of multiple genes/pathway involved in apoptosis, cell invasion, and adhesion**

Curcumin controls the activities of additional molecular targets that regulate cell adhesion, apoptosis, and invasion. In this context, curcumin has been revealed to be an extremely potent inhibitor of TNF-α-induced expression of intracellular cell adhesion molecules-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin in human umbilical vein endothelial cells. By apparently inhibiting the induction of steady-state transcription levels of ICAM-1, VCAM-1 and E-selectin, curcumin may be interfering detrimentally with the the TNF-α-induced signaling event at an early stage. Additionally, curcumin has been shown as anticancer, chemosensitive, and radiosensitive which effects via activation of p53 and simultaneous downregulation of MDM2 oncogene expression via the PI3K/mTOR/ETS2 pathway in human prostate cancer (PC3) and colon cancer (HT-29) cell lines (Li et al., 2007), and induce apoptosis and nuclear translocation and activation of p53 in human neuroblastoma cells (Liontas and Yeger, 2004).

**Curcumin regulating activities of several enzymes mediated tumor growth**

Moreover with direct regulating the gene expression, curcumin also interferes the activities of enzymes that mediate in tumorogenesis and proliferation of cells. Curcumin blocks fibrosis in anti-Thy1 glomerulonephritis through upregulation of hemoxygenase-1 (HO-1) gene expression, suggesting antifibrotic
effects with glomerular disease (Gaedeke et al., 2005). Similarly, curcumin can also induce HO-1 expression through the generation of reactive oxygen species, p38 activation, and phosphatase inhibition (McNally et al., 2007).

Curcumin can also suppress tumor cell growth through its effects on Ras protein pathways. In order to extend their biological activity of Ras proteins, isoprenylated at a conserved cysteine residue near the carboxyl terminus (Cys-186 in mammalian Ras p21 proteins). Previous studies have indicated that most likely farnesyl pyrophosphate, donates this isoprenyl group and inhibitors of the mevalonate pathway might be able to block the transforming effects of Ras oncogene expression. By evaluating the role for curcumin and its derivatives, they seems to be strongly mechanism for suppression of cellular growth (Aggarwal et al., 2006).

In another investigations, it has been shown that curcumin remarkably inhibit the activity of xanthine oxidase (XO) in vitro in PMA-treated NIH3T3 cells. Induction of XO activity is considered a major cause of PMA-mediated tumor promotion, and curcumin’s marked ability to inhibit PMA increases, such activity appears as direct inactivation of the XO protein (Shishodia et al., 2007).

**Preclinical studies of curcumin**

**Curcumin is a potent chemopreventive agent**

Curcumin can suppress the tumorigenic activity of a wide variety of carcinogens like cancers of colon, duodenum, esophagus, fore stomach, stomach, liver, breast, leukemia, oral cavity, and prostrate. A study in mice, it has found that curcumin inhibited 7, 12-dimethylbenz[a]anthracene (DMBA)-initiated and 12-O-tetra-decanoylphorbol-13-acetate (TPA)-promoted skin tumor formation (Sreepriya and Bali, 2006). Curcumin has ability to suppress mammary tumor-initiating activity of DMBA (Sigletary et al., 1996) and formation of DMBA-DNA adducts in female rats (Deshpande et al., 1998) which indicated chemopreventive activity against progression stage of colon carcinogenesis (Kawamori et al., 1999).
Study in rodents showed that curcumin was able to inhibit the development of N-methyl-N'‐nitro-N-nitrosoguanidine (MNNG)-induced stomach cancer (Ikezaki et al., 2001), an effect mediated in part by an ability by suppressing the proliferation of *Helicobacter pylori* major causes for gastric cancer (Mahady et al., 2002).

**Pharmacokinetic and pharmacodynamic studies of curcumin**

The pharmacokinetics and pharmacodynamics properties of curcumin has been also widely investigated. Wahlstrom and Blennow (1978) performed first study in Sprague-Dawley rats to investigate the uptake, distribution, and excretion of curcumin. When curcumin was administered orally at dose of 1g/kg, then about 75% of ingested curcumin was excreted through faeces and there was negligible in urine. Curcumin was poorly absorbed by blood plasma and biliary systems from the gut. Curcumin doses up to 5 g/kg did not produce toxic effects. After thirty minute oral administration of curcumin, 90% of its amount was distributed in stomach and small intestine, but after 24 h lastly only 1% remained there (Ravindranath and Chandrasekhara, 1980). On the basis of several findings, Perkins et al. (2002) suggested that about 1.6 g of curcumin is essential for efficacy in humans. Oral dosing of curcumin in human at 4-8 g resulted to at highest (0.41-1.75 µM) plasma levels (Cheng et al., 2001). A study conducted on 15 patients feded with 36-180 mg curcumin orally daily up to 4 months, no curcumin metabolites were detected in blood and urine, whereas it was appeared in the faeces (Sharma et al., 2001). Previously, it has been reported that curcumin at doses of 8 g/day or lower was not toxic and detected peak in serum at 1-2 h (0.51±0.11 µM at 4 g, 0.63±0.06 at 6 g, and 1.77±1.87 µM at 8 g) and released through nonurinary routes within 12 h (Cheng et al., 2001).
*Helicobacter pylori*

*Helicobacter pylori* (H. pylori) is a gram negative pathogenic bacteria which causes gastric diseases like gastritis and peptic ulcer in human. This is one of a major risk factor in development of gastric cancer (Marshall, 1994; Gerhad et al., 2002). In 1994, *Helicobacter pylori* has been considered as a class I carcinogen by World Health Organisation.

Earlier several studies have shown high rate of homologous gene recombination in *Helicobacter pylori*. *Helicobacter pylori* has been subdivided phylogenetically into distinct biogeographic populations and subpopulation with certain geographical distributions and ancient human migrations was reflected (You et al., 2012). East Asian group of population are differentiated from the European group on the basis of biochemical events like mechanism of electron transfer and redox reactions, which indicated model of adaptive evolution as well as selection through proteome diversification and modulation of translational fidelity (Mikihiko et al., 2011).

The half of the world’s populations are infected with *Helicobacter pylori* depending on socio-economic status (Suerbaum and Michetti, 2002). The transmission mode of this bacteria are oral ingestion and then colonisation in the gastric mucosa (Suerbaum and Michetti, 2002). *H. pylori* infection causes developing of various clinical diseases from inflammation in gastric mucosa, then development of ulcers, and after prolongation turn into gastric cancer. *H. pylori* infected persons show risk of 10-fold increase in development of gastric cancer (Kusters et al., 2006). Moreover, this bacteria may also produce deficiencies of iron and vitamin B12, idiopathic thrombocytopenic purpura (ITP), and growth retardation in children. The colonisation of *Helicobacter pylori* occurs in childhood and exist throughout life, but its high activity causes diseases in adults (Blaser and Atherton, 2004). Members of *Helicobacter* genus have been classified into two groups on the basis of site of infection, these are (i) gastric *Helicobacter* species that colonisation in the gastric region of the gastrointestinal (GI) tract (ii) colonisation of the enterohepatic *Helicobacters* in the small intestine and hepatobiliary regions of the GI tract.
Some of the enterohepatic *Helicobacter* species cause an inflammatory diseases in animal model, which is similar to Inflammatory Bowel Disease (IBD) in humans (Shomer et al., 1997; Foltz et al., 1998).

**Relationship between *Helicobacter pylori* and Humans:**

As it has been reported that *Helicobacter* inhabits in the gastrointestinal tract of many mammals and birds, therefore, they are often called as gastric *Helicobacter* species. These species are mostly host-specific, and indicate the concept of co-evolution with their hosts. Falush et al. (2001) have reported that *Helicobacter pylori* and its hosts share a common ancestor as compared with nucleotide sequences of different strains and by measuring maximal in vivo mutation rates. It has been seen that genetic diversity of *Helicobacter pylori* strains among different regions decreases corresponding to decreasing genetic diversity in human population depending on distance in East Africa (Linz et al., 2007). This indicates that *H. pylori* has coevolved with humans since their point exodus from Africa 60,000 years ago and likely exists throughout their evolutionary process.

**H. pylori colonization and adaptations in human:**

According to previous reports *Helicobacter pylori* colonizes in human stomach since childhood and persist throughout our lives by acquiring adaptation (Banatvala et al., 1993). Mechanism of perfect adaptation of *H. pylori* to the niche and capability to evade the human immune response have been explained (Banatvala et al., 1993). Its spiral shape and flagella allow to corkscrew through the gastric mucus gel, and numerous adhensin enable selective adherence to the epithelium. *H. pylori* has enormous mechanism to proliferate against gastric acid (Sachs et al., 2003); as evidenced with 15% of its protein content comprises cytoplasmic urease. At the external pH is less than 6.5, a specific channel opens in the bacterial cytoplasmic membrane, allowing ingress of urea (Weeks et al., 2000). The ammonia produced by urea hydrolysis neutralizes the periplasm and allow maintenance of the cytoplasmic membrane potential (Weeks et al., 2000).

Like human commensal bacteria, *H. pylori* has evolved specific mechanism to avoid stimulating the immune response for example, innate immune recognition
by several TLRs (Toll-like receptors) attenuation (Backhed et al., 2003). Despite this, its colonisation is associated with inflammatory cell infiltration into the gastric mucosa termed gastritis. *H. pylori* strains induce varying degrees of gastritis, reflecting their individual abilities to interact with host and possess “host interaction” (also known as virulence) factors (Banatvala et al., 1993). But how these factors result to the diseases required to be better understood, although the benefit to *H. pylori* by possessing them remain less clear. One possibility is that the epithelial changes caused by *H. pylori* (directly and perhaps also through inflammation) allow to increase nutrient delivery to the bacterium. Additionally, host interactions may induce niches modification, resulting to better conditions for survival of competing bacteria.

Death of Emperor Napoleon Bonaparte was occurred on 5th May 1821. His death made mysterious and has enthralled historians for years. The autopsy report of his personal physician late Dr Francesco Antommarchi and the English physician indicated that cause of his death was due to gastric cancer (Antommarchi, 1825; Hindmarsh and Corso 1998; Lemaire et al., 2001). This report came after examination the non-neoplastic and prepyloric ulcer in stomach of Napoleon and that begins the history of chronic gastritis by *Helicobacter pylori*, which might have caused a risk of gastric cancer. Further this was also explained that the cause was based on his usual diet like salt-preserved foods, roasted meats, and few fresh fruits and vegetables.

On other hand, Sokoloff (1938) had published autopsy report of Napoleon’s father (Charles Bonaparte) with stating that his father also died by gastric cancer, and concluded that Napoleon could have had a familial predisposition for stomach cancer due to hereditary transmission (Sokoloff, 1938). Although autopsies of other family member of Napoleon could not prove the basis of heredity reason but only suspected and medico history and symptoms only (Lemaire et al., 2001; Tulard 2005). On the basis of present science, tt has been assumed that a general predisposition, may have accompanied by a polymorphism in the interleukin 1β gene, which has highly increased the risk for gastric cancer in individual infected with *Helicobacter pylori* (El-Omar, 2001). *H. pylori* infection in Napoleon cannot be excluded but still remains in the realm of speculation.
Epidemiology and Natural History

Since years, the prevalence of *H. pylori* infection and the incidence of gastric cancer is gradually decreasing, more accentuated in wealthy Western societies. Consequently, the genotypes of bacteria are also varying such as virulent strains localised in population showing risk for gastric in comparison to lower risk of population. Sometimes more than one type of *H. pylori* strain are colonized in gastric mucosa express different types of virulence. The healthy changes in societies are reflecting simultaneously decrease in occurring of cancer rate and it has been indicated that risk of infection related with economic status (Plummer et al., 2004). Mostly prominent factors prevailing are improved sanitation around home, decreasing family size, less house-hold crowding, changes in dietary habits such as less salt consumption and more intake of fruits and fresh vegetables, improvements in refrigeration at home and food safety transportation equipment and control of infectious diseases. Other factors also are vigorous use of antibiotics to cure other disease resulting to development of resistance in *H. pylori*.

Generally in beginning *H. pylori* infection are asymptomatic, but symptoms appear later as heartburn, dyspepsia, nausea, vomiting or halitosis. The hematemesis or melena may be found in subjects who suffer from erosive gastritis or ulcers. Initially, antrum is infected and eventually may spread proximally to the corpus. Prolonged or severe infection may develop ulcer or atrophy (loss of glandular tissues). Where subjects of duodenal ulcers are not at increased risk of gastric cancer (Uemura et al., 2001) and subjects with gastric ulcers are at high risk for gastric cancer (Uemura et al., 2001; Hansson et al., 1996). Intestinal-type adenocarcinoma (Lauren, 1965), which is more frequent in populations have more incidence rate for gastric cancer and at a multistep and multifactorial process depending on environmental conditions (*H. pylori* infection, diet and smoking) have vital role for etiopathogenetic. In contrast, population at low risk of gastric cancer have shown frequent diffuse-type adenocarcinoma at high risk. Where environmental factors play less important role than genetic factors. Anatomically, stomach adenocarcinoma are comprised into noncardia (more prominent in worldwide) and cardia cancer.
which are also associated with *H. pylori* (Dawsey et al., 2002; Kamangar et al., 2007).

**Pathogenesis of Helicobacter pylori**

The two main pathways involve in progression of gastric cancer by *Helicobacter pylori*: (i) the indirect action of *Helicobacter pylori* through inflammation in gastric epithelial cells, and (ii) the direct action of bacteria with epithelial cells. Several studies have shown that the gastritis play vital role in development of gastric cancer. In this sense another factor may be *Helicobacter pylori* alters directly cell function by several bacterial factors, such as CagA virulent factor (Segal et al., 1999; Tsutsumi et al., 2006). Although the relationship between the two above pathways are not clearly demonstrated perhaps they work together to promote gastric cancer development.

**Urease: A favourable key factor for buffering stomach pH**

Prior to the bacterium moves inside the mucus layer it makes with the contact with intestinal epithelial cells for surviving in the extreme pH of the stomach lumen. The seven genes are responsible for biosynthesis of the urease enzyme (Labigne et al., 1991; Achtman and Suerbaum, 2001). These genes encode UreA (26.5 kDa) and UreB (60.3 kDa) and five other proteins which is required for the uptake of Ni+2 for urease activity (Achtman and Suerbaum, 2001). In presence of urease, urea hydrolysed into ammonia that buffers the cytosol and periplasm and helps forming neutral layer around the bacterial surface. *Helicobacter pylori* uptakes urea through a proton-gated channel which opens at lower pH. At neutral pH, the urea channels are blocked to avoid surrounding of excessive alkaline environment.

The authors also have described that the extracytoplasmic urease activity formed a cloud of ammonia around the cells leading to the formation of a neutral pH microenvironment (Stingl et al., 2002).

*Helicobacter pylori* with defective urease activity do not colonize the stomach gnotobiotics (Eaton et al., 1991). This strong evidences elucidated that urease is essential for colonisation and survival of *Helicobacter pylori*. In addition,
urease activity also produce toxicity by formation of ammonium, which damage the cells. Moreover, ammonium also react with the reactive intermediates generated by the neutrophil myeloperoxidases to form carcinogenic agents associated with *Helicobacter pylori* for adenocarcinoma (Megruad et al., 1992).

**Role of flagella and attachment and motility to the host cells**

At the circumstances of avoiding prolonged exposure to acid and getting discharged in the intestine, *Helicobacter pylori* moves to the stomach epithelial cell line within the thick mucous layer. *Helicobacter pylori* has 4-6 lophotrichous flagella which act as a propeller to travel to the viscous mucus layer like a screw into a cork. The chemotactic movements require different factors like urea and bicarbonate ions (Yoshiyama et al., 1993). Whereas non-motile bacteria as a mutant cannot colonize inside the stomach.

Several factors have been identified in *Helicobacter pylori* that play role as virulent for disease development. Among them cytoxin-associated gene (cagA) and the vacuolating cytotoxin A (vacA) gene are most prominent factors. The 40 kb DNA of cag pathogenicity island acquired by cagA gene and encodes a type IV secretion system and provide a passage for the translocation of cagA protein into the epithelial cells (Censini et al., 1996). The presence of CagA protein has been reported in some strain and suggested that only, 50-60% acquired by *H. pylori* in Western countries and >90% of isolates from East Asian Countries, which are based on acquisition of DNA from other bacteria (Censini et al., 1996). The cagA-positive *H. pylori* has been recognised on the basis of its role in development of peptic ulcer (Covacci et al., 1993; Tham et al., 2001) as well as gastric adenocarcinoma (Blaser et al., 1995; Parsonnet et al., 1997). The mechanism has been suggested as CagA translocation into gastric epithelial cell, and phosphorylating the tyrosine residues of five amino acids (EPIYA) motifs resulting into multiple alternation in cells (Azuma, 2004). It has been reported that the EPIYA-repeat region of CagA is extensively divergent among in species of *Helicobacter pylori* and is composed of a combinations of four segments: EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D. Most of *H. pylori*'s CagA protein were isolated from Western countries regions which possess EPIYA-A, EPIYA-B, and one of the three copies of the
EPIYA-C motifs. The isolated strains from East Asian countries EPIYA-D motif is replaced with EPIYA-C motifs which induces more severe changes in cell, gastric atrophy, inflammation, and gastric cancer in comparison to strains from Western countries (Azuma, 2004; Satomi et al., 2006). Most strain from Western region having multiple EPIYA-C sites in CagA depending on phosphorylation for biological activity (Higashi et al., 2002; Naito et al., 2006).

The another virulent factor vacA gene encodes a vacuolating cytotoxin secreted by H. pylori and damages epithelial cells (Cover, 1996). The vacA gene is found in all strains with two different variable regions (Atherton et al., 1995). The s region encodes single peptide s1 with subtypes s1a, s1b, and s1c or s2 allele. The m (middle) region exists as m1 or m2 with two subtypes m2a or m2b alleles (van Doorn et al., 1998). The cytotoxin associated pathogenesis has been determined on the basis s and m region alleles combination (Atherton et al., 1995). The strain vacA m1-type are associated with severe damages of gastric epithelial (Atherton et al., 1997) and produces gastric ulcer or carcinoma, which is more active as compared to m2 strains (Figueiredo et al., 2001). Mainly, vacA s1, vacA m1, and cagA-positive genotypes of Helicobacter pylori are found to be associated with higher degree of inflammation, atrophy, and intestinal metaplasia (Nogueira et al., 2001).

Human epidermal growth factor receptor2 (HER2)

Human epidermal growth factor receptor 2 (HER2) encodes a 185-kDa glycoprotein with transmembrane tyrosine kinase receptor, p185. It belongs to the EGFR (epidermal growth factor receptor) and plays a key role in signal transduction of growth factor. The overexpression and amplification of HER2 in solid tumor leads to oncogenic transformation and tumorogenesis (Koeppen, et al., 2001). Presently, the HER2 as singaling pathways proved to be major therapeutic target.

HER2 expression/amplification have been demonstrated in breast cancer, associated with aggressive tumor growth, poor prognosis, and increased risk of disease recurrence (Slamon et al., 1987; Revillion et al., 1998). In addition, due to the overexpression and amplification, it has been used as predictive
marker for targeted therapy analysed by the monoclonal antibody trastuzumab, or a fully humanised anti-HER2 monoclonal antibody (Dowsett et al., 2007).

In 1986, HER2 overexpression was first explained in gastric cancer (Jorgensen, 2010). HER2 expression has been also reported in ovarian, lung, endometrium, salivary duct, colon, and gastric cancer (Jewell et al., 2006; Tapia et al., 2007). A growth inhibitory effect of trastuzumab has been reported in vitro in cultured human gastric tumor cell lines overexpressing HER2 and in vivo in these cell lines growing as xenografts similar to breast cancer (Tanner et al., 2005).

HER2 has no specific ligand, that require co-receptor to form dimers with EGFR, HER3, or HER4; the heterodimers between HER2 and these receptors showed more efficiency for translating mitogenic signals than the homodimers, and are synergetic for cellular transformation (Pinkas-Kramarski et al., 1996; Graus-Porta et al., 1997). Moreover, many reports have explained that overexpression of HER2 acts direct role in pathogenesis and tumor development due to: (1) HER2 establishment in healthy cell leads to malignant transformation, (2) development of breast tumor due to HER2 overexpression in transgenic mice, and (3) the monoclonal antibodies implication with HER2 subdue the tumor growth and indication of overexpression of the receptors (Slamon et al., 2001). The several types of human cancers have indicated the amplification and overexpression of HER2 gene, but showed poor prognosis in patients (Koeppen et al., 2001). HER2 expression in gastric cancer has been demonstrated by several methods (Allgayer et al., 2000; Garcia et al., 2000). For example, amplification (Hirono et al., 1995) and immunohistochemical (Yonemura et al., 1989) methods are used to determine the HER2 expression in cancerous tissues related even at the poor prognosis in gastric cancer patients.

On the experimental basis, human epidermal growth factor receptor 2 (HER2) has been shown to be encoded by c-erbB-2 proto-oncogene located on chromosome 17q12-q21 region (Edge and Compton, 2010). The amplification of HER2 occurs 15% to 25% of gastric carcinoma and overall prevalence of 19% was described according to meta-analysis data (Ross and McKenna, 2001).
The correlation of HER2 status with prognosis in gastric cancer remains controversial according to some studies which based on HER2 expression/amplification. (Ross and McKenna, 2001; Latif et al., 2002). However, according to earlier reports HER2 overexpression is highly related to differentiated or intestinal type gastric cancers, and showed a better prognosis than undifferentiated or diffuse-type cancers (Latif et al., 2002).

The immunohistochemical method has been utilized for HER2-targeted therapy and detection of HER2 overexpression and amplification of HER2 gene in cancerous patients by fluorescence in situ hybridization (FISH) in primary tissue (Wolff et al., 2006). It has been suggested that the antigenic profile of primary tumors may be different from that metastatic diseases. A discrepancy between the primary tumor and distant metastases was observed in 7% to 20% of cases (Regitnig et al., 2004; Zidan et al., 2005). Therefore, the reassessment of HER2 status at the time of disease progression might help to optimize the treatment by identifying additional patients who could be benefited from trastuzumab or other therapeutic approaches against HER2. Here biopsy of the metastatic lesion may not be an appreciate option because of its location.

An alternative, invasive tissue analysis could be better to determine the serum HER2 status. Concerning to above, when extracellular domain of the HER2 receptor is released after cleavage by ADMA (a disintegrin and metalloproteinase) metalloproteinases that casuse in serum using ELISA (Carney et al., 2003). Elevated serum HER2 levels are highly correlated with HER2 over-expression and amplification in tumor tissue (Molina et al., 1996). Another possibility of the evaluation of HER2 status can be on the basis of immunohistochemistry or FISH of circulating tumor cells (CTCs), which are frequently detected in the blood of metastatic breast cancer patients (cell-based assay) (Meng et al., 2004). These methods are helpful for detection of unknown HER2 status patients in with recurrent breast cancer.

**Pathological diagnosis of HER2-positive gastric cancer**

Alternatively methodology of pathological diagnosis of gastric cancer is based on fixing of biopsy sample in formaldehyde and paraffin-embedded as standardized by World Health Organization in 2010. The ToGA trial method a
reliable HER2 testing in patients of gastric cancer has been suggested for anti-HER2 treatment (Bang et al., 2010).

Now-a-days, many challenges are arising related with diagnosis of HER2 gastric cancer. The clinical stage may not be confirmed with biopsies of gastric cancer. while at advanced stage of gastric cancer more samples are needed for multiple information including pathology. Pathologists apply the different approaches i. e. assessment of HER2 status is more important than correct neoplasm classification. Biopsies samples are sectioned using tissue-sparing technique (Gomez-Martin et al., 2011). It has been seen that intestinal-type adenocarcinoma are with high HER2-positive than mixed- or diffuse-type neoplasms (Gomez-Martin et al., 2012). HER2 overexpression/amplification has been associated with high proliferative index neoplasms and histological hepatoid subtypes (Giuffre et al., 2012).

Still challenge are persisting related with methodological using of IHC and ISH. Results are obtained easily after using different ISH techniques, because interpretation is easier with silver-enhanced ISH (SISH), which is an advantage for diagnosing focal amplification in very small biopsies (Boers et al., 2011; Ruschoff et al., 2012). But the question remain whether SISH underestimates or overestimates the HER2 for example chromosome 17 ratio comparison with two-colour FISH is still controversial (Stenzinger et al., 2012).

Biological associated with the heterogeneity of HER2 alterations in gastric cancer is still a pathological challenge. Intratumoural heterogeneity has been shown to be of prognostic significance (Yoon et al., 2012) and is the main reason for discordance between IHC and FISH, in biopsies and resection specimens (Yang et al., 2012). Although high concordance has been shown for HER2 status between primary and paired metastases (Kim et al., 2011). However, endoscopic testing of biopsies has been favoured, because theoretically covers a larger area than one section from a surgical specimens. In some studies, more IHC 3+ cases were significantly seen with biopsies than resection specimens (Bang et al., 2010; Lee et al., 2011). Recently, it was found that combining with the analysis of diagnostic biopsies and surgical specimens enables the optimization the selection of trastuzumab-eligible patients.
in cases of metastatic relapse, particularly in previously neoadjuvant chemotherapy-responding patients (Watson et al., 2013).

**Bioinformatics**

**Bioinformatics in drug discovery**

Drug discovery a stepwise process in which new candidates are discovered. Generally, pharmaceutical companies follow well-established pharmacology and chemistry-based drug discovery approaches, but some time face various difficulties in finding new drugs (Iskar et al., 2012). In the highly competitive “winner takes all” pharmaceutical industry, the first company to patent a new chemical entity (NCE i.e., new drug candidate) for a specific treatment takes all the spoils, and there leaving by other competitors to wait for patent expirations to partake in the largesse (Iskar et al., 2012). Nowadays, therefore, Pharmaceutical companies are investing heavily to implement those approaches which are potential to accelerate the drug development process. With increasing pressure to generate more and more drugs in a short period of time with low risk has resulted remarkable interest in bioinformatics (Ortega et al., 2012).

Presently, the sources of leading drugs in the pharmaceutical industry have changed a lot. Since 1970’s large empirically-based screening programs has been taken up but proved to be less important for the drug industry but with advancement in technique knowledge base increased to design rational drug (Kubinyi, 1995). In this era, leading compounds from sources were discovered by using both in vitro and in vivo screening assays from other than primary in vitro screens. Lead sources coming from natural products are becoming popular but varying with opinion as: clinical observations of drug side effects (Kubinyi, 1995); published unexamined patents; published reports in scientific journals and collaborations with academic investigators. Most of these lead sources showed the common theme ‘chemical lead’ with considerable scientific investigation but not identified as a drug lead. On the view point of physical property, the most poorly compounds in an analogue series were being eliminated and most often the starting lead compound were carrying in a range of consistent physical properties due to previous historical record of discovery of orally active compounds.

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Several leading compounds have pharmacological properties and plays a pivotal role in discovery of drugs. Lead compounds such as morphine, quinine, atropine, curcumin, etc. isolated from natural sources are used clinically against various diseases. Although, most of them need structural modification for enhancement of their biological activities and lowering the side effects. For developing an orally active compound, certain properties of lead compound has been taken into consideration under Lipinski’s rule of five of Veber’s parameters that helped pharmaceutical scientists to select the suitable candidates or to reject with a low probability of success (Lipinski et al., 1997; Veber et al., 2002). Overall, computer based (in silico) molecular modelling (bioinformatics and cheminformatics) has been quite useful for this purpose, because they are extremely fast and cost efficient and can be also applied even when compounds are not physically available (Terstappen and Reggiani, 2001).

**Role of Bioinformatics in Natural drug discovery**

Historically, the discovery of novel drugs from source of natural products had been always related with chemistry and pharmacology. Most of the natural products have most active ingredients which are for application in medicines. Natural products as source of medicine have been applied since ancient periods before the advent of high-throughput screening (HTS) and the post-genomic era. It was demonstrated that more than 80% of drugs are made from natural products (Sneader, 1996). Most of drugs from natural products were found to be closely compliant with Lipinski’s Rule of Five and recommended for orally intake considering with high molecular weights, more rotatable bonds and more stereogenic centres, although they had low logP values. This has been also indicated that natural products are more readily absorbed than synthetic drugs (Ganesan, 2008).

Drug-discovery programs have been established start with reorganisation of drug targets. Such targets are biomolecules as receptor proteins, enzymes and ion channels. The initial steps of drug target validation have been done *in vitro* and in animal models and finally it was validated clinically in humans. Although most of the process of early pharmaceutical research relied
predominantly on experimental work in the laboratory, but at present computer is becoming increasingly important. One of the most emerging areas in silico approaches are being used in discovery of drug targets. Currently, on the above circumstances bioinformatics is becoming very essential in areas of all the science researches (Terstappen and Reggiani, 2001).

It has been suggested that plant metabolites are a promising inhibitor of tumor, cancer, and other severe diseases, while silico study has showed limitation in discovery of natural lead compounds (Gordaliza, 2007) due to lack of availability of 3D database which require for docking studies, their viability and complex structure. While naturally isolated compounds are most preferred source for discovery of drugs (Mangal et al., 2013). Analysis of natural products as source of new drugs over the period 1981-2002 indicates that only 39% of the 877 New Chemical Entities (NCEs) can be classified as truly synthetic in origin, among which in the area of anti-infective and anticancer is 70% and 67% of the NCEs are naturally derived or inspired respectively (Cragg and Newman, 2005).

**In silico library design and virtual screening**

In silico two basics applications have been categorised: diversity and structure-based design for virtual screening of compounds. Diversity design aims to select a small hub-library from a larger compounds in such a way that the full range of chemical diversity can be best represented (Gorse and Lahana, 2000). The different computational methods used for compounds selection are based on similar clustering, grid-like partitioning of chemical space or the application of genetic algorithms (Drie and Lajiness, 1998). The results of such in silico diversity selections (in silico screening) are considered as smaller sub-libraries of manageable size with a high degree of chemical diversity and subjected to HTS in vitro.

While structure-based library design has been proved to be biased due to structural requirements for the activity on a particular target and prior information of the target structure (e.g. X-ray or nuclear magnetic resonance). The goal is to select from existing compound libraries or to design compounds with three-dimensional complementarity (i.e. shape, size and physicochemical
properties) as the target-binding site. In the latter case, new approaches have directly guided a design of virtual combinatorial libraries, which are first screened in silico for target complementarity to reduce the number of compounds which are synthesised and tested in vitro. The combination of structure-based design and combinatorial chemistry, called ‘combinatorial docking’ (Boehm and Stahl, 2000). Besides above, different computational tools and methods have been also reviewed elsewhere (Li et al., 1998). It was expected that the hit-rate (rate of active compounds on the target in a dose-dependent manner) of focused libraries can be higher than that of diversity screening for example using of protease cathepsin D showed the hit-rate of the focused approach which was more than twice higher than that of diversity screening (Kick et al., 1997).

Most X-ray structures available are for enzymes, whereas membrane proteins as receptors and ion channels are exceedingly difficult to crystallize. For such difficult targets, a focused approach has been employed when ligands (natural or synthetics) are recognised as the actual target or for the respective target class. In silico screening of the pharmacophore against virtual libraries has been carried out and interesting compounds synthesized for HTS using combinatorial chemistry. Now both diversity and structure-based screening can be performed in an iterative manner. In this case, the results of in vitro HTS can be analysed in silico by using programs such as SCAM (statistical classification of the activities of molecules)] (Rusinko, 1999) to derive rules that can be used for the rational selection of further molecules to be tested in vitro in future.

For implication of bioinformatics, structural information of biological molecules are readily available in the Protein Data Bank (PDB), http://www.pdb.org. Upto April 2009, the PDB had shown approximately 57,000 experimental protein structures, that has been divided into ~3500 families, consisting of nearly 1100 unique folds (http://scop.mrc-lmb.cam.ac.uk./scop/count.html#scope-1.73). At present the number of nonredundant amino acid sequences entries are around 408,000 (http://www.expasy.org/sprot/), and there is huge gap between known annotated sequences and available 3D structures. Despite the rapid growth of the PDB, the structural novelty of proteins number (defined as
<25% sequence identity between 2 structures) deposited in the PDB has remained constant since 1992 (Levitt, 2007). At least one representative structure from protein families without having structural information according to experimental data and several structural genomics (SG) projects have been initiated, which contributed roughly 50% of the novel structures (as <30% sequence identity) in the PDB over the past five years (Levitt, 2007; Lundstrom, 2007). The above experimental contribution proved to be helpful in bioinformatics analysis in future line of action.
Fig. 1. Mechanism of Helicobacter pylori