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Recent advances in the synthesis, chemical transformations and pharmacological studies of some important dietary spice's constituents

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Abstract:

Chemoprevention, a relatively new and promising strategy to prevent various human disorders, is defined as the use of natural dietary compounds and/or synthetic substances to block, inhibit, reverse, or retard the process of their occurrence. Spices are valued not only as food adjuncts to enhance the sensory quality of food but also for their medicinal properties. The spices such as dietary garlic, onion, fenugreek, red pepper, turmeric, and ginger have been proven to be effective hypocholesterolemics in experimentally induced hypercholesterolemia. The hypolipidemic potential of fenugreek in diabetic subjects and of garlic and onion in humans with induced lipemia has been demonstrated. Capsaicin and curcumin - the bioactive compounds of red pepper and turmeric respectively - are documented to be efficacious at doses comparable to usual human intake. This review explores recent work on the chemical transformations, synthesis of analogues and their pharmacological uses of key phytochemicals from some important Indian dietary spices such as *Curcuma longa* (Turmeric or Haldi), *Zingiber officinale* (Ginger), *Piper nigrum* (Black pepper), *Allium sativum* and *Allium cepa* (Garlic and Onion), *Crocus sativus* (Saffron or Kesar) and *Trigonella foenum-graecum* (Fenugreek or Methi) during the period 2008 to 2013. It also contains an overview of the global and Indian nutraceutical market.

Introduction

Today, many ethnic cuisines are recognized for their reliance on “signature” herbs and spices for example turmeric in Indian; basil, garlic, and oregano in Italy and Greek; and lemongrass, ginger, cilantro, and chili peppers in Thai food. Satia-Abouta *et al* ^[1]

reported that the cuisines of Asia, South-East Asia, and the Mediterranean are perceived by many to be healthier than the

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typical Western diet. For instance, one of the highest rates of gastric cancer mortality in Europe occurs in Italy; yet rates are recognized to vary markedly across regions in the country.^[2] Buiatti *et al* ^[2] determined that individuals who consumed more meats, salted fish, cold cuts and seasoned cheeses had the highest risk for gastric cancer, while those consuming more fresh fruit, raw vegetables, onion, garlic, and spices were associated with lower risk. In Asian countries, the consumption of curcumin, a component of curry powders, turmeric and mustard, along with low meat intake, have been reported to be factors linked to a lower incidence of colon cancer.^[3]

Diets high in fat may increase the risk of heart disease and some forms of cancer. On the contrary, increased intake of fruits, vegetables, herbs and some of their constituents reduces risks and may even prevent some diseases. These beneficial effects keep on prompting research-community to carry out more and more studies; as a result the number of research publications related to dietary substances is significantly increased. Curcumin is one such example (Figure1).

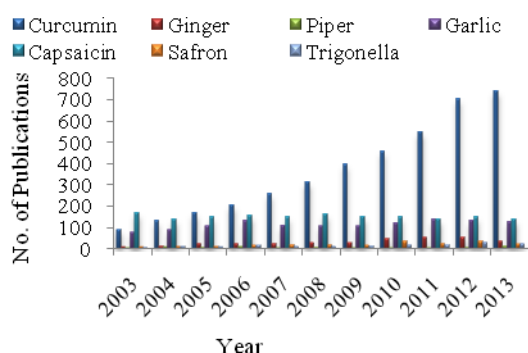


Figure 1: Year-wise number of publications on some dietary spices. (Data were collected by using curcumin, ginger, piper, garlic, capsaicin, safron, trigonella as key words in pubmed accessed on December 24th, 2013).

Presently, strategies to improve health are a driving factor for market growth within the food and beverage industry.^[4] Interestingly, it has been reported that about 77% of U.S.

households claim they are trying to reduce their risk of heart disease and cancer.^[5] Americans between the ages of 36 and 55 are increasingly interested in adopting healthy eating behaviours and are gravitating towards ethnic cuisines, such as Asian and Mediterranean, based on the perceived health benefits associated with these types of cuisines.^[6] Although some ethnic cuisines may be considered to be healthier than others, overall food consumption patterns, as well as food preparation techniques, are also likely equally important.

Nutraceutical industry

Natural dietary supplements have major contribution to nutraceuticals. All the major nutraceutical markets in the world are keeping on focusing towards natural healthy dietary supplements.

The global nutraceuticals market is estimated at about \$151 billion in 2011. According to Global Nutraceutical Industry: Investing in Healthy Living report by 2016, it is estimated to reach nearly \$207 billion (Figure 2), a projected compound annual growth rate (CAGR) of 6.5% between 2011 and 2016. Functional beverages market is expected to experience the highest growth, at a compound annual growth rate (CAGR) of 8.8% during the 5-year period from 2011 to 2016. This sector is expected to be worth \$57 billion in 2011 and nearly \$87 billion in 2016.

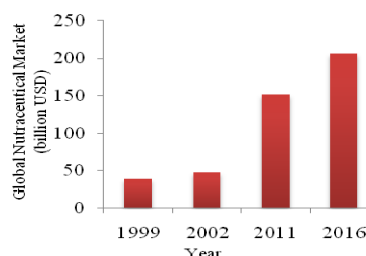


Figure 2: Growth of global nutraceutical market (Global Nutraceutical Industry: Investing in Healthy Living report by 2016)

Nutraceutical food market is the second largest market, generating an estimated \$49 billion in 2011. This should reach \$67 billion in 2016, for a CAGR of 6.4%.

United States (US) nutraceutical market

In 2010, the United State nutraceutical market stood at US \$ 50.4 billion and was by far the largest nutraceutical market in the world (Figure 3). It is fast approaching maturity in the dietary supplements segment, while functional food and beverages are quickly catching up the maturity. The growth rate of dietary supplements segment and functional food and beverages segment were growing roughly at a 3.1 and 5.6% rate. Currently, companies in the US are focusing towards natural nutraceutical ingredients in their product offering, mainly due to the increasing consumer demand for all-natural, non-modified functional ingredients.

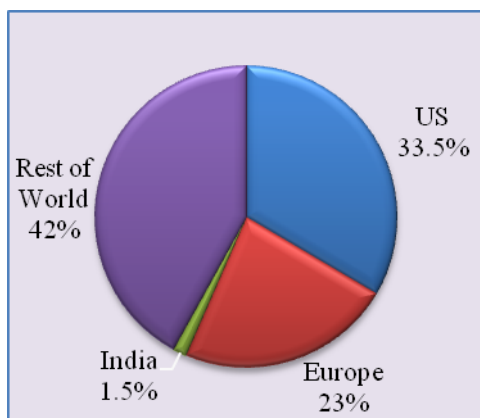


Figure 3: Global contribution of different nutraceutical markets in 2010 (Global Nutraceutical Industry: Investing in Healthy Living report by 2016)

European nutraceutical market

The total European nutraceutical industry was valued at US \$ 35 billion in 2010 (Figure 3). Europe's focus within the nutraceutical industry is on innovation and new product development, resulting in

increasing R&D spends in the sector, up from 0.24% of the industry revenue in 2004 to between 0.8 to 1% in 2010. Companies in Europe believe that product and ingredient innovation is the way forward for the nutraceutical industry. Germany, Netherlands and Sweden have emerged as the key nutraceutical innovation hubs in Europe, while Great Britain and Spain have emerged as key test markets for new products.

Indian nutraceutical market

In 2010, the Indian nutraceutical industry was estimated at US \$ 2 billion, roughly 1.5% of the global nutraceutical industry (Figure 3). It is currently a nascent market trying to incorporate traditional herbal ingredients (usually ayurvedic) into the nutraceutical portfolio. Key example is the chyawanprash supplements market in India, which stood at US \$74.5 million in 2010. The existence of alternative medicine in India, and the Indian consumer's belief in them, could provide a platform for the nutraceutical industry to capitalize on. The Indian consumer's awareness about conventional nutraceutical ingredients is severely limited, and nutraceutical manufacturers need to spread awareness about their products to the Indian masses.

Chemoprevention by natural dietary compounds

Chemoprevention is defined as the use of natural dietary compounds or synthetic substances to inhibit or prevent the process of their occurrence. It is a relatively new and promising strategy to prevent cancer and many other disorders. The chemopreventive effects of these natural dietary compounds are believed due to their effects on different biochemical processes such as antioxidative, anti-inflammatory activity, induction of phase II enzymes, apoptosis, and cell-cycle arrest.

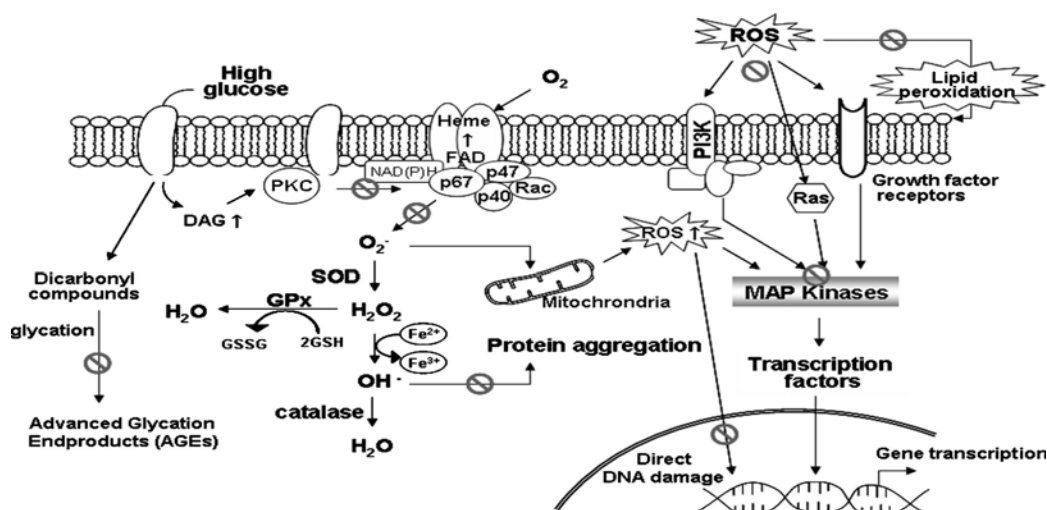


Figure 4: Possible mechanisms of ROS production, oxidative damage and targets for food bioactive compounds. In mammalian cells, the ROS are generated during irradiation, metal-catalyzed reactions, enzymatic reactions and mitochondria-catalyzed electron transport reactions. The various protective mechanisms are marked with a Φ ^[9]

Both laboratory and clinical studies have presented evidences that support the crucial role of reactive oxygen species (ROS) in the pathophysiology associated with atherosclerosis, neurodegenerative diseases, and all stages of carcinogenesis.^[7] Oxidative stress – a consequence of imbalance between the generation and removal of ROS, resulting in potential cell damage. These ROS include free radicals such as hydroxyl radical, peroxy radical, superoxide anion radical, and other reactive species, such as hydrogen peroxide and singlet oxygen, generated as a result of natural physiological processes (e.g., mitochondrial electron transport, exercise), environmental stimuli (e.g., ionizing radiation from the sun), environmental pollutants, changed atmospheric conditions (e.g., hypoxia), and lifestyle stressors (e.g., cigarette smoke and excess alcohol consumption). However, increasing evidence in both clinical and experimental studies suggests a role for various reactive carbonyl species (RCS)^[8] produced during lipid peroxidation (mainly of α,β -unsaturated aldehydes), or generated as a consequence of the reaction of reducing sugars, such as glyoxal and methylglyoxal, or their oxidation products

with lysine residues of protein. Most of the biological effects of intermediate RCS are attributed to their capacity to react with the nucleophilic sites of proteins, forming advanced lipoxidation end products (ALEs) and advanced glycation end products (AGEs) (Figure 4).

Reactive oxygen Species are generally removed from the body by different enzymes such as superoxide dismutase, catalase, glutathione peroxidase and antioxidants, which can exist endogenously in the body or be consumed from natural dietary sources. Oxidative damage can occur to macromolecules such as proteins, DNA and lipids. DNA base alterations, strand breakage and mutations are the major problems associated with free radical attacks on DNA. This damage can be stopped, reduced and even reversed with antioxidant supplementation. Antioxidant protection from damage due to free radicals is vital for the integrity of cellular structures and macromolecules.^[9]

Natural dietary foods and herbs that possess anticancer activity and can be used for cancer-chemoprevention include garlic, soybeans, cabbage, ginger, licorice, onions, flax, turmeric, cruciferous vegetables, tomatoes, peppers, brown rice,

wheat and the umbelliferous vegetables such as carrots, celery and parsley. Natural products and their isolated chemical constituents have been shown to possess strong chemopreventive activity in animal models.^[10] The effects of nutraceuticals on apoptotic pathways, cellular signaling pathways, and different targets in different diseases mean that they could be helpful starting points in the design and development of novel cancer preventive agents.

Safety and Upper Limits

Herbs and spices fall under “Generally Recognized As Safe” (GRAS) category by the FDA, at least at concentrations commonly found in foods; however, many herbs, spices, and their bioactive components, are being investigated for potential disease prevention and treatment at concentrations which may exceed that in food preparations. It is therefore essential to identify any potential safety concerns associated with the use of various dosages which range from doses commonly used for culinary purposes to those used for medicinal purposes since there are often unclear boundaries between the various uses of herbs and spices.

Several herbs and spices of culinary origin which have included in the “approved” monographs, are caraway oil and seed, cardamom seed, cinnamon bark, cloves, coriander seed, dill seed, fennel oil and seed, garlic, ginger root, licorice root, mint oil, onion, paprika, parsley herb and root, peppermint leaf and oil, rosemary, sage, thyme, turmeric root, and white mustard seed. The majority of the uses for these herbs and spices relate to dyspepsia and other gastrointestinal disturbances so do not pertain directly to cancer prevention, but the monographs do provide dosage guidelines, which indirectly may serve to guide researchers in establishing safe human dosages for herbs and spices

under investigation for cancer treatment and prevention purposes.

Health Effects

A number of phytochemical constituents isolated from dietary herbs and spices have been found to be potential modifiers of fatal disease like cancer and other lifestyle diseases. Despite rapidly growing experimental evidence towards chemopreventive properties of herbs and spices, very little data are available regarding their actual dietary intake levels. Consequently, researchers are limited to compare the effective exposures of dietary spices, and their bioactive secondary metabolites from experimental studies, which have performed on animal models, using approximate human intake levels. Furthermore, information on the uptake, distribution, and excretion of most non-nutritive dietary components is sparse and little data related to human blood levels of these compounds has been published in the scientific literature.^[11] Despite these limitations, research indicates that herbs and spices, or their bioactive components, may act alone or in concert to reduce disease risk through their anti-microbial, anti-oxidant, and anti-tumorigenic properties, as well as their direct suppressive effects, on different biochemical pathways.

(1)

Curcuma longa (Turmeric or Haldi)

Introduction

Phytochemicals are naturally occurring substances found in plants as secondary metabolites. There has been considerable public and scientific interest in the use of phytochemicals derived from dietary herbs and spices to combat human diseases, especially the two commonest killers in the developed world, cardiovascular disease and cancer. The dried ground rhizome of the perennial herb

Curcuma longa-Linn., called turmeric in English, haldi in Hindi and ukon in Japanese, has been used in Asian medicine since the second millenium BC (Figure 5).^[12] Its utility is referred to in the ancient Hindu scripture, the Ayurveda. In addition to its aromatic, stimulant and colouring properties in the diet, turmeric is mixed with other natural compounds such as slaked lime and has been



Figure 5: *Curcuma longa* (from Koehler's Medicinal-Plants)

used topically as a treatment for wounds, inflammation and tumours. In contrast to the maximum dietary consumption of 1.5 g per person per day in certain South East Asian communities, smaller quantities of turmeric tend to be used for medicinal purposes.^[13] The use of turmeric as a colouring, food preservative and flavouring is global.

Chemical constituents Curcumin

Curcumin (diferuloylmethane) is a low molecular weight polyphenol. It was chemically characterised in 1910 for the first time and is the most active constituent of and comprises 2–8% of most turmeric preparations.^[14,15] *Curcuma spp.* contain turmerin (a water-soluble peptide), essential oils (such as turmerones, atlantones and zingiberene) and curcuminoids including curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3, 5-dione]. Curcuminoids are a group of phenolic compounds derived from the roots of *Curcuma spp.* (Zingiberaceae).

Chemistry of Curcumin

Curcumin is a bis- α,β -unsaturated β -diketone. As such, curcumin exists in equilibrium with its enol tautomer (Figure 6). The bis-keto form predominates in acidic and neutral aqueous solutions and in the cell membrane.^[16] At pH 3–7, curcumin acts as a potent H-atom donor.^[17] This is due to the presence of an active methylene (CH_2) flanked by two keto groups and the C–H bonds on this carbon are very weak due to delocalisation of the unpaired electron on the adjacent oxygens. In contrast, above pH 8, the enolate form of the heptadienone chain predominates, and curcumin acts mainly as an electron donor, a mechanism more typical for the scavenging activity of phenolic antioxidants.^[17] Curcumin is relatively insoluble in water, but soluble in acetone, dimethylsulphoxide (DMSO) and ethanol.

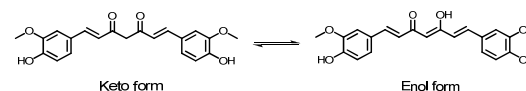


Figure 6: Structure of Curcumin (1)

Curcumin is unstable at basic pH, and degrades within 30 min to trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin.^[18] The presence of foetal calf serum or human blood, or addition of antioxidants such as ascorbic acid, N-acetylcysteine or glutathione, completely blocks this degradation in culture media or phosphate buffer above pH 7. Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1h. Other investigators have also found that curcumin is more stable in cell culture medium containing 10% foetal calf serum or in human blood, with less than 20% decomposition within 1 h compared to 90% within 30 min in serum-free medium.^[16] The complex kinetics of pH-dependent degradation of curcumin in aqueous solution was first reported by

Tonnesen and Karlsen.^[19] These investigators studied the photochemical stability of curcumin, and offered the first suggestion of its potential antimicrobial activity by photosensitization.^[20] Curcumin has a molecular weight of 368.37 and a melting point of 183°C. Commercial grade curcumin contains the curcuminoids desmethoxycurcumin (10–20%) and bisdesmethoxycurcumin (less than 5%). On ultraviolet–visible spectrophotometric investigation, maximum light absorption of curcumin occurs at 420 nm. Studies in preclinical models of carcinogenesis have demonstrated that commercial grade curcumin has the same inhibitory effects as pure curcumin.^[21,22] It is not known whether essential oils derived from *Curcuma spp.* have similar intrinsic activity to curcumin.^[23]

Curcumin and its analogues

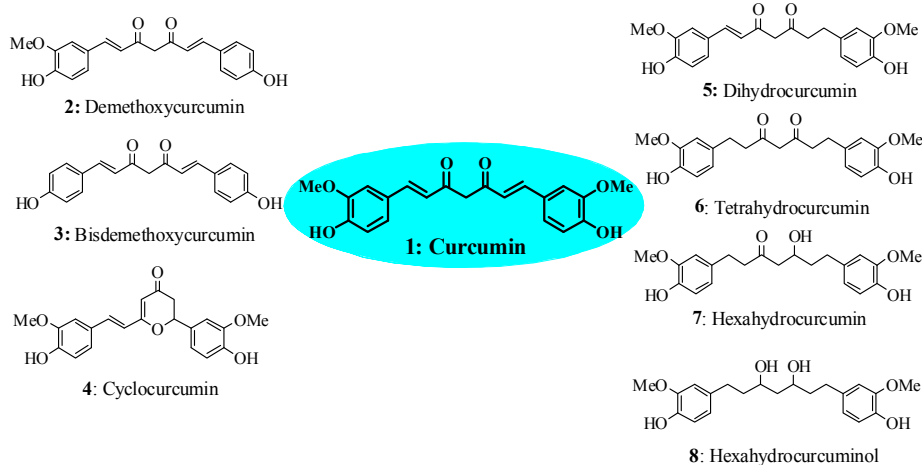


Figure 7: Natural analogs from *Curcuma longa* (turmeric)

Apart from the above reported chemical constituents, novel terpecurcumins A–I (**9–17**; Figure 8), secondary metabolites have been isolated from the rhizomes of *C. longa* (turmeric). They were derived from the hybridization of curcuminoids and bisabolanes. The structures and absolute configurations of **9–17** were elucidated on the basis of extensive spectroscopic data analysis,

Natural analogues

Curcuminoids having anticancer activity include mainly curcumin, demethoxycurcumin, bisdesmethoxycurcumin, and bisabolane sesquiterpenoids including arturmerone, α -turmerone, and β -turmerone. Particularly, curcumin is a new antineoplastic agent, which is currently in phase II clinical trials for the treatment of colorectal and pancreatic cancer. A total of 27 curcuminoids and 38 sesquiterpenoids have been isolated from turmeric, and some of them show even more potent cytotoxic activities than curcumin (Figure 7).^[24] Recently, four hybrids conjugating the curcumin skeleton with a bisabolane-type sesquiterpene were isolated from turmeric, although the absolute configurations and biological significance were not defined.^[25]

including NMR and electronic circular dichroism spectra.^[26] Among these compounds **12**, **14**, and **15** showed higher cytotoxic activities (IC_{50} , 10.3–19.4 μ M) than curcumin (IC_{50} , 31.3–49.2 μ M) itself against human cancer cell lines (A549, HepG2, and MDA-MB-231)

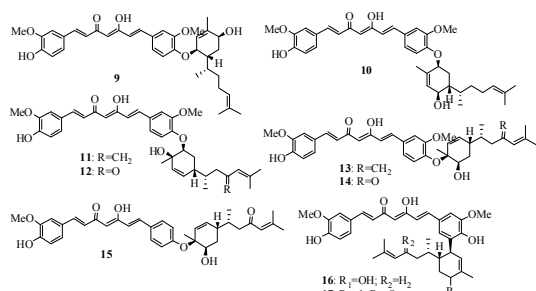


Figure 8: Terpecurcumins A-I (9-17) from *C. longa*

Synthetic derivatives and analogues

Curcumin and its analogues have been the subject of much interest due to its pleiotropic pharmacology. Recent computational studies have shown that the optimized structure of curcumin is coplanar with the enol-form as the stable ground state.^[27] The characteristic structural features of curcumin include two *O*-methoxy phenol units, two enone moieties, a 1,3-diketone and a reactive methylene. These diverse structural features provide many interesting sites for chemical modifications (Figure 9).

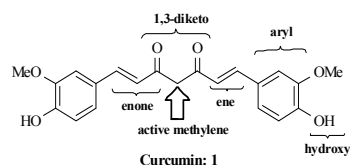


Figure 9: Possible sites for chemical modification in curcumin

The curcumin derivatives are generally synthesized by derivatization, starting from curcumin. For example, the phenolic hydroxy group may be acylated, alkylated, glycosylated, and amino acylated. The methoxy groups may be demethylated to hydroxy groups.^[28] The reactive methylene group of the linker may be acylated or alkylated or substituted by an arylidene group (Ar-CH)^[29], thereby introducing substituents on the C7 chain.

Qiu *et al.* (2010) have synthesized several arylidene derivatives (60-75) of curcumin and evaluated them for their improved NF- κ B inhibition activity (Figure 10a-b).^[30]

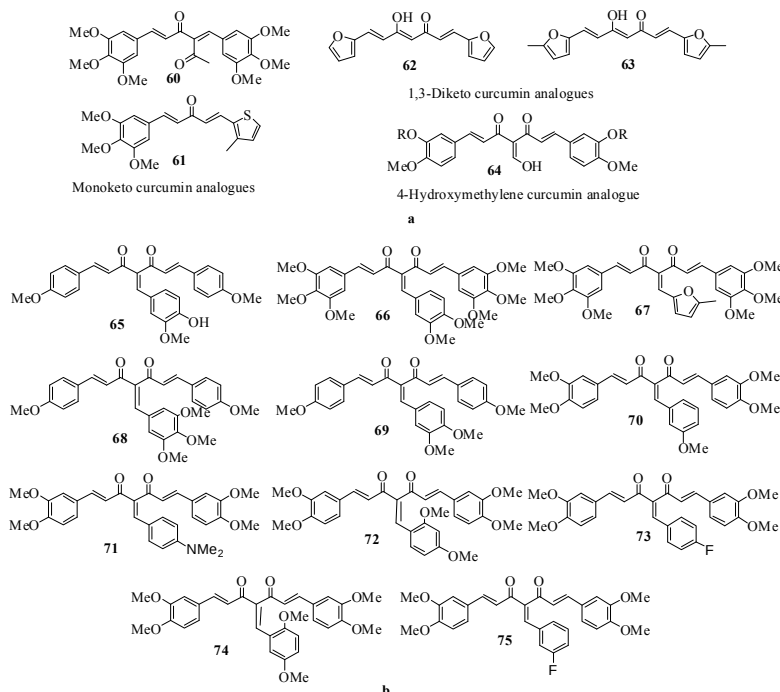


Figure 10 (a): Structures of monoketone curcumin analogs (60-61); 1,3-diketones curcumin analogs (62-63); 4-hydroxymethylene curcumin analogues (64); **(b):** Structures of 4-arylidene curcumin analogues (65-75)

Ferrari *et al.* (2011) have synthesized new curcumin analogs (ester; **76-79** and acids; **80-83** Figure 11) and tested for anticancer activity against different cancer cell lines (human ovarian carcinoma cells –2008, A2780, C13*, and A2780/CP, and human colon carcinoma cells HCT116 and LoVo).^[31] Most of ester derivatives showed IC₅₀ values lower than curcumin and exhibited selectivity against colon carcinoma cells. Especially they were found extremely active after 24 h exposure showing enhanced inhibitory effect on cell viability. The best performances of ester curcuminoids may attributed to their high lipophilicity that favours a greater and faster cellular uptake

overcoming their apparently higher instability in physiological condition.

Most recently Ruan *et al.* (2012) have synthesized a series of resveratrol-curcumin hybrids (Scheme 1) and evaluated for their antiproliferative activity against three cancer cell lines including murine melanoma B16-F10, human hepatoma HepG2 and human lung carcinoma A549.^[32] Among these, compound **86** displayed the most potent in vitro antiproliferative activity against B16-F10 with IC₅₀ value of 0.71 µg/mL. Compound **86** also exhibited good tubulin polymerization inhibitory activity with IC₅₀ value of 1.45 µg/mL.

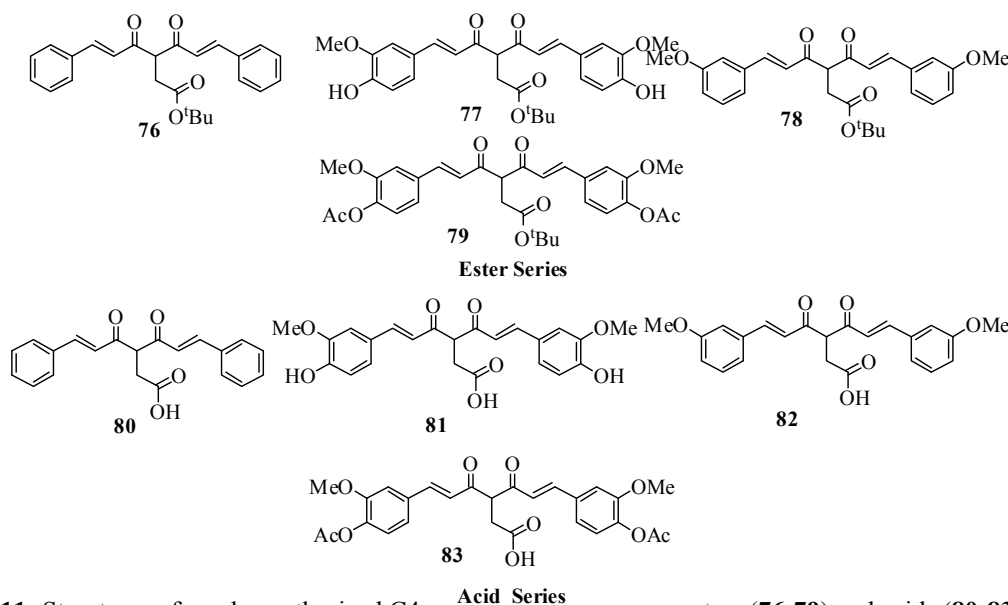
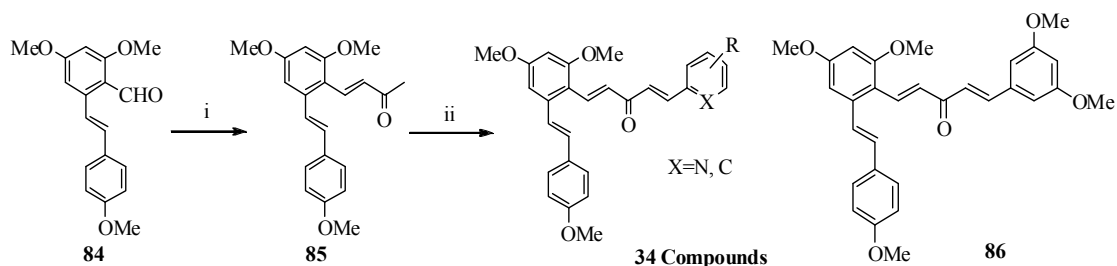


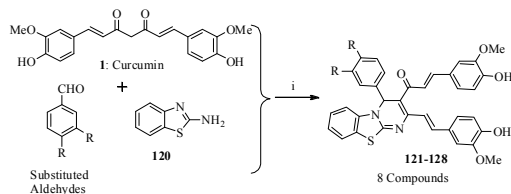
Figure 11: Structures of newly synthesized C4 esters (**76-79**) and acids (**80-83**)



Scheme 1: Reagents and conditions: (i) NaOH (2 M), acetone, rt; (ii) NaOEt, EtOH, substituted benzaldehydes or pyridinecarbaldehydes, rt.

Sahu *et al.* (2012) reported one-pot, simple, efficient synthesis for 4H-pyrimido[2,1-b]benzothiazole, pyrazole and benzylidene derivatives of curcumin under solvent and solvent free conditions in microwave with good yield.^[33] These derivatives of curcumin have been synthesized by three component reaction using, substituted aromatic aldehydes and 2-amino benzothiazole (**120**) and pyridine under solvent and solvent free conditions (Scheme 2). The synthesized compounds were evaluated for their antibacterial activity against gram-positive and gram-negative bacteria viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus* and *Providencia rettgeri* and antifungal activity against fungi viz. *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*.

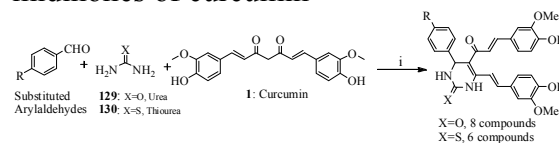
Scheme 2:



Reaction Conditions (i) refluxing in methanol at 60-65 °C using pyridine as catalyst

Lal *et al.* (2012) synthesized 3,4-Dihydropyrimidinones of curcumin in excellent yield by multi-component one-pot condensation of curcumin, substituted aromatic aldehydes and urea (**129**)/thiourea (**130**) under solvent free conditions using $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ catalyst (Scheme 3).^[34] All the synthesized compounds were evaluated for their synergistic antimicrobial (antibacterial and antifungal) and in vitro cytotoxicity against three human cancer cell lines Hep-G2, HCT-116 and QG-56. Compounds **131-133** and **139-140** (Figure 12) showed interesting antimicrobial and cytotoxic activity as compared to curcumin.

Scheme 3: Synthesis of 3,4-Dihydropyrimidinones of curcumin



Reagents and conditions (i) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, No Solvent, 80°C, 90min

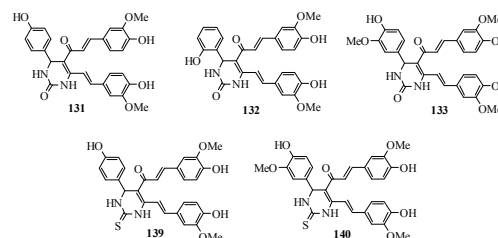
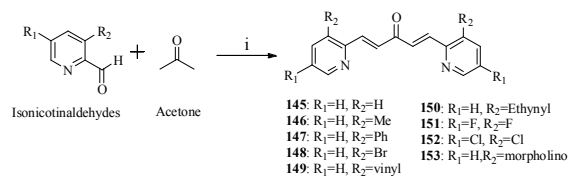


Figure 12: Active compounds

Bin Cao *et al.* (2012) synthesized novel pyridinyl analogs of dibenzylideneacetone (pyr-dba) by the condensation of substituted isonicotinaldehyde and acetone in the presence of K_2CO_3 in toluene-EtOH- H_2O solvent system (Scheme 4).^[35] The resulting compounds (**145-153**) functioned as the enone analogs of curcumin and efficiently inhibited the activation of NF- κB and the growth of colorectal carcinoma HCT116 p53+/+ cells as well as the HIV-1 IN-LEDGF/p75 interaction.

Scheme 4: Synthesis of pyridinyl analogs of dibenzylideneacetone

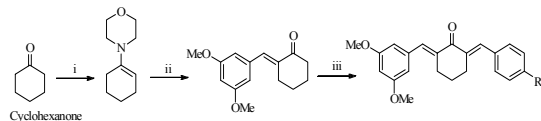


Reagents and conditions (i) K_2CO_3 , Toluene-Ethanol- H_2O , 70°C, 12hr

Xu *et al.* (2013) have synthesized novel α,β -unsaturated cyclohexanone analogs, as EGFR inhibitors based on the curcumin core structure (Scheme 5).^[36] These compounds exhibited potent antiproliferative activity in two human tumor cell lines (Hep G2 and B16-F10). Among them, compounds **150** and **151** displayed the most potent EGFR inhibitory

activity ($IC_{50} = 0.43\mu M$ and $1.54\mu M$, respectively) (Figure 13).

Scheme 5: Synthesis of α,β -unsaturated cyclohexanone analogs



Reagents and conditions (i) Morpholine, reflux, 5hr (ii) 3,5-dimethoxybenzaldehyde, reflux, 8h, followed by HCl, rt, 2h; (iii) substituted benzaldehydes, NaOH or HCl, rt, 30min

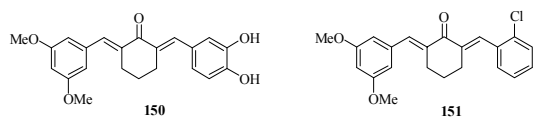
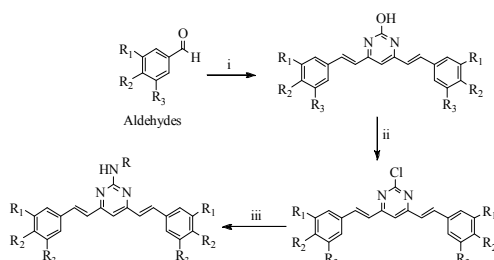


Figure 13: Active α,β -unsaturated cyclohexanone analogs

Qiu *et al.* (2013) have synthesized six (**152-157**) novel pyrimidine substituted curcumin analogs (Scheme 6, Figure 14) with or without hydroxyl group in which the critical plane conjugate structure of curcumin was retained while the chemical stability and solubility were improved.^[37] The cell viability tests indicated that IC_{50} of the three curcumin analogs containing hydroxyl group was 3 to 8-fold lower than that of the three analogs without hydroxyl group in two colon cancer cell lines tested.

Scheme 6: Synthesis of pyrimidine substituted curcumin analogs



Reagents and conditions (i) 4,6-dimethyl-2-hydroxyl-pyrimidine hydrochloride, HCl, EtOH, PhMe, 110°C, 36 h, (ii) $POCl_3$, DIPEA, 110°C, 12h (iii) Appropriate amine, EtOH, 90°C, 12h

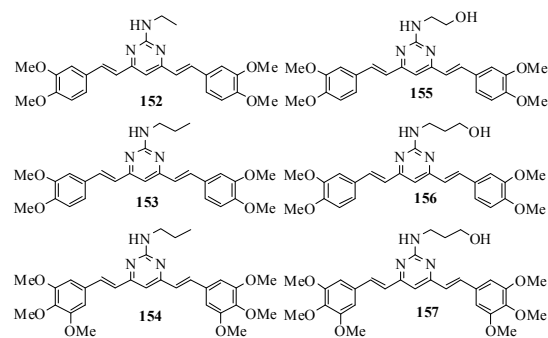
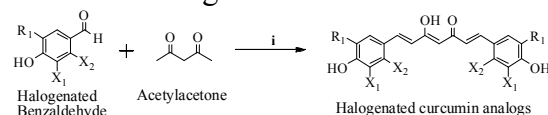


Figure 14: Chemical structures of non-hydroxylated (**152-154**) and hydroxylated pyrimidine substituted curcumin analogs (**155-157**)

Batie *et al.* (2013) synthesized halogenated curcumin analogs (**158-162**) as nuclear receptor specific agonists (Scheme 7, Figure 15).^[38]

Scheme 7: Synthesis of halogenated curcumin analogs



Reagents and conditions: (i) $B(Bu)_3$, $N(Bu)_3$, B_2O_3 , Ethylacetate, 40°C, 24hr

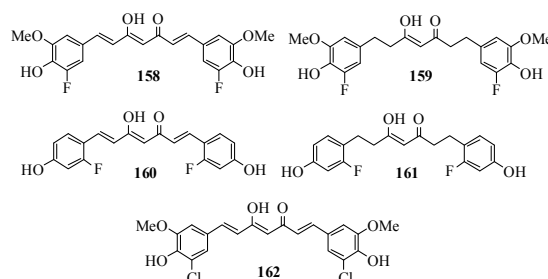


Figure 15: Structures of synthesized halogenated curcumin analogs

Pharmacology of Curcumin

Curcumin (diferuloylmethane), the active ingredient in turmeric (*C. longa*), has highly diversified pharmacological profile with anti-inflammatory, antioxidant, chemopreventive, chemosensitization radiosensitization and anticancer activities. The pleiotropic pharmacological profile attributed to curcumin come from its complex molecular structure and chemistry, as well

as its ability to influence multiple signalling molecules.

The unique structural features of curcumin viz. bis - α,β -unsaturated β - diketone, two methoxy groups, two phenolic hydroxy groups and two double-conjugated bonds might play an essential role in activities assigned to curcumin. Various preclinical and clinical studies have demonstrated that curcumin has potential therapeutic value against most chronic diseases including neoplastic, neurological, cardiovascular, pulmonary, metabolic and psychological diseases.

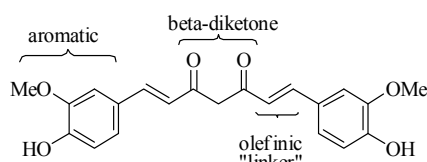


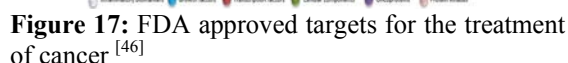
Figure 16: Functional regions of Curcumin

Curcumin is a functionally labile molecule with the potential to modulate the biological activity of a number of signalling molecules either indirectly or directly by binding through covalent, non-covalent hydrophobic, and hydrogen bonding interactions. Curcumin has two hydrophobic phenyl domains that are connected by a flexible C7 linker, and molecular docking studies have found that curcumin can adopt different necessary conformations required for maximizing hydrophobic contacts with the target protein to which it is bound. For example, the phenyl rings of curcumin can participate in π - π van der Waals interactions with aromatic amino acid side chains. Within generally hydrophobic structure of Curcumin, the phenolic and carbonyl functional groups, which are located on the ends and in the center of the molecule respectively, can participate in hydrogen bonding with a target macromolecule. This structure provides a strong and directed electrostatic interaction to increase favourable free energies of association. Infact curcumin is found to bind to DNA not through intercalation of

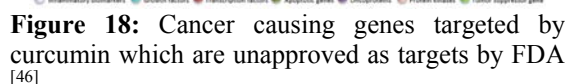
the phenyl rings but through hydrogen bonding interactions with the minor groove in AT-rich regions.^[39,40] Owing to its β -diketone moiety, curcumin undergoes keto-enol tautomerism and exists entirely in the enol form both in solution and in solid phase.^[41,42] This keto-enol tautomerization provides curcumin with additional chemical functionality. The predominant enol form allows the midsection of the molecule to both donate and accept hydrogen bonds. The enol form also makes an ideal chelator of positively charged metals, which are often found in the active sites of target proteins.^[43] Finally, the keto-enol tautomerization allows curcumin to act as a Michael acceptor to nucleophilic attack, and curcumin has been found to bind covalently to nucleophilic cysteine sulfhydryls and the selenocysteine moiety.^[44,45] The combination of all these structural features gives curcumin many possible mechanisms to interact with target proteins.

Curcumin and Cancer

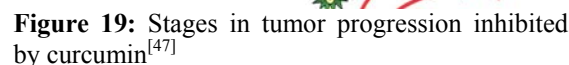
The peculiar structural features of curcumin are infact, responsible for its pleiotropic pharmacology. Curcumin can be classified as both a semiselective and a cytotoxic agent as it interacts with the targets of the semiselective agents (growth factors and oncoproteins) and the cytotoxic agents (cellular components). Curcumin has been shown to suppress transformation, proliferation, and metastasis of tumors. These effects are mediated through its regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes (Figure 17).



There are, however, many curcumin targets for which no FDA-approved drug is available (Figure 18).^[46]



Bioavailability of Curcumin



Problems

(i) Serum Concentration and Tissue Distribution

One of the major observations related to curcumin studies involves the

observation of extremely low concentration in serum and tissues.^[49,50]

(ii) Metabolites

Various studies have evaluated the metabolism of curcumin in rodents and in humans. Once absorbed, curcumin is subjected to conjugations like sulfation and glucuronidation at various tissue sites.^[51,52] Major biliary metabolites of curcumin are glucuronides of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC) in rats. A minor biliary metabolite was dihydroferulic acid together with traces of ferulic acid.^[53]

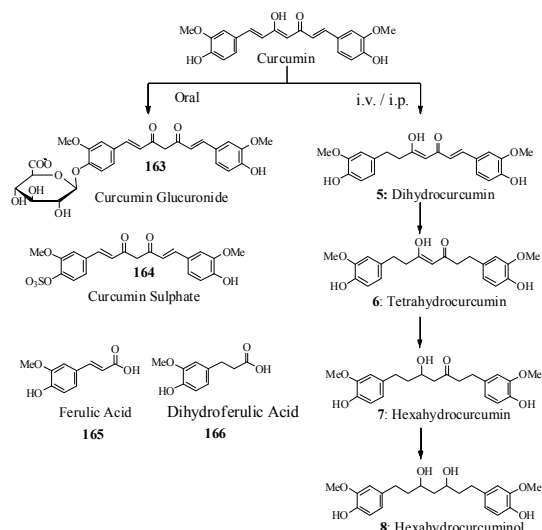


Figure 20: Structure of metabolites of curcumin

In addition to glucuronides, sulfate conjugates were found in the urine of curcumin treated rats. Hydrolysis of plasma samples with glucuronidase by Pan et al. Showed that 99% of curcumin in plasma was present as glucuronide conjugates.^[54]

(iii) Half-Life

Systemic elimination or clearance of curcumin from the body is also an important factor, which determines its relative biological activity. Studies have revealed that the major route of elimination of the radio labelled products was through feces; urinary excretion was very low regardless of the dose.^[53,55,56]

Promises

The absorption, biodistribution, metabolism, and elimination studies of curcumin have, unfortunately, shown only poor absorption, rapid metabolism, and elimination of curcumin as major reasons for poor bioavailability of this interesting polyphenolic compound.

(i) Adjuvants

Adjuvants are the chemical agents which assist an administered drug to get its enhanced concentration in blood plasma i.e. bioavailability. Piperine, a known inhibitor of hepatic and intestinal glucuronidation, was combined with curcumin and simultaneously administered in rats and healthy human volunteers and found to be much effective. The effect of piperine on bioavailability of curcumin has been shown to be much greater in humans than in rats.^[56]

(ii) Nanoparticles

Recently, targeted and triggered drug delivery systems accompanied by nanoparticle technology have emerged as prominent solutions to the bioavailability of therapeutic agents with poor tissue distribution.

Most recently Tiwari *et al.* (2013) have been reported that curcumin encapsulated PLGA nanoparticles (Cur-PLGA-NPs) potently induced neural stem cells (NSC) proliferation and neuronal differentiation in vitro and in the hippocampus and subventricular zone of adult rats, as compared to uncoated bulk curcumin.^[57] Cur-PLGA-NPs has been found to induce neurogenesis by internalization into the hippocampal neural stem cells (NSC). Cur-PLGA-NPs significantly increased expression of genes involved in cell proliferation (reelin, nestin, and Pax6) and neuronal differentiation (neurogenin, neuroD1, neuregulin, neuroligin, and Stat3). Curcumin nanoparticles increased

neuronal differentiation by activating the Wnt/ β -catenin pathway, involved in regulation of neurogenesis

(iii) Liposomes, Micelles, and Phospholipid Complexes

Liposomes are excellent drug delivery systems since they can carry both hydrophilic and hydrophobic molecules. Li *et al.* investigated the *in vitro* and *in vivo* antitumor activity of liposomal curcumin against human pancreatic carcinoma cells and demonstrated that liposomal curcumin inhibits pancreatic carcinoma growth and, in addition, exhibits antiangiogenic effects. Liposomal curcumin suppressed the pancreatic carcinoma growth in murine xenograft models and inhibited tumor angiogenesis.^[58]

Micelles and phospholipid complexes can improve the gastrointestinal absorption of natural drugs, thereby giving higher plasma levels and lower kinetic elimination resulting in improved bioavailability. The intestinal absorption of curcumin and micellar curcumin formulation with phospholipid and bile salt was evaluated using an *in vitro* model consisting of everted rat intestinal sacs. This study supports biological transformation of curcumin during absorption.

(iv) Derivative and Analogues

The chemical structure of curcumin plays a crucial role in its biological activity. For example, isomerization has been proved to have an influence on antioxidant activity of curcumin.^[59] Therefore researchers hope to achieve improved biological activity of curcumin by structural modifications. Various studies dealing with the enhanced biological activity of curcumin derivatives and/or analogues can be found in the literature.

(2)

Zingiber officinale (Ginger)

Introduction

Ginger, the rhizome of *Zingiber officinale*, (Figure 21) is one of the most widely used species of the ginger family (*Zingiberaceae*) around the world. It is a common condiment for various foods and beverages with a long history of medicinal use dating back 2,500 years in China and India.



Figure. 21: *Zingiber officinale*
(Rhizome)

Chemical Constituents

Ginger contains bioactive compounds such as gingerols, shogaols, paradols, and zingerones.^[60] Of the ginger compounds, [6]-gingerol, the most abundant bioactive compound in ginger, has been extensively studied for its various pharmacological effects including anti-inflammatory, analgesic, antipyretic, chemopreventive, and antioxidant properties.^[61] Interestingly, recent studies have demonstrated that 6-shogaol, a minor component of ginger, might be more biologically active than [6]-gingerol.^[62]

Bhattarai *et al.*^[63] have recently reported that 6-gingerol can be degraded to [6]-shogaol in a model system such as acidic conditions and at high temperature. Ginger contains a number of pungent constituents and active ingredients. Steam distillation of powdered ginger produces ginger oil, which contains a high proportion of sesquiterpene hydrocarbons, predominantly zingiberene.^[64]

Jolad *et al.* (2005) identified 88 compounds from a methylene chloride extract of commercially processed dry ginger, *Z. officinale* by gas chromatography-mass spectrometry (GCMS).^[65] Out of these 88 compounds, 45 were previously reported by the same group from fresh ginger^[66], 12 cited

elsewhere in the literature and the rest (31) were new. These compounds include mainly paradols, gingerols, isogingerols, shogaols, gingerdiones, 1-dehydro-[6]-gingerdiones, gingerdiols.

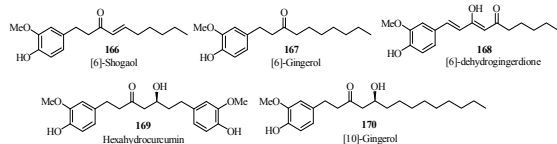
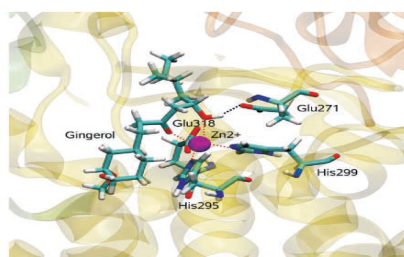
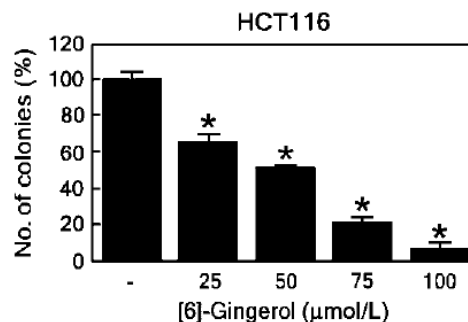


Figure 22: Structure of 166-170

Chen *et al.* (2009) isolated five pure phenolic compounds (**168-170**) along with **166** and **167** from the rhizomes of *Z. officinale* (ginger) (Figure 22) and investigated for their antiallergic potency in rat basophilic leukemia (RBL-2H3) cells model.^[67] The data obtained suggest that ginger rhizomes harbour potent compounds capable of inhibiting allergic reactions and may be useful for the treatment and prevention of allergic diseases. [6]-gingerol has been found to suppress colon cancer growth by targeting leukotriene A₄ hydrolase.^[68] The leukotriene A₄ hydrolase (LTA₄H) protein is regarded as a relevant target for cancer therapy. They revealed the LTA₄H as a potential target of [6]-gingerol by in silico prediction using a reverse-docking approach. [6]-Gingerol specifically binds with Glu271 of LTA₄H (Figure 23). They also proved their prediction by showing [6]-gingerol mediated cancer cell growth suppression by inhibiting LTA₄H activity in HCT116 colorectal cancer cells.



(A)



(B)

Figure 23: **A)** Close-up view of the interactions of [6]-Gingerol within the LTA₄H catalytic site. The hydrogen bond between the ligand and Glu271 (both in stick representation) is shown as a dotted black line. **B)** [6]-Gingerol inhibits anchorage-independent growth of HCT116 cells.

Novel glucosides of gingerdiol were isolated from fresh ginger and their antioxidant activity was evaluated (Figure 24).^[69]

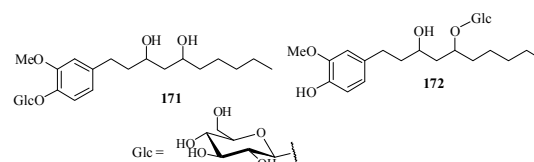


Figure 24: Glucosides of gingerdiol

Nievergelt *et al.* (2010) identified nine compounds (**173-182**) that interact with the human serotonin 5-HT_{1A} receptor with significant to moderate binding affinities (Figure 25).^[70] The serotonin 5-HT_{1A} receptor (5-HT_{1A}R) is a G-protein coupled receptor widely expressed in the central nervous system (CNS), where it is involved in the modulation of mood and emotion and of different behavioural responses, including thermoregulation, sleep, feeding, aggression, and anxiety.

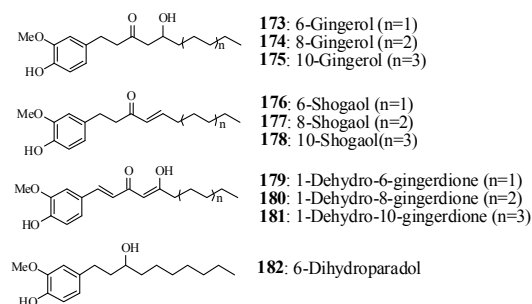


Figure 25: Compounds showing binding affinities to human serotonin 5-HT_{1A} receptor

Juan *et al.* (2011) isolated three unprecedented purine-containing compounds (**183-185**), named [6]-, [8]-, and [10]-zingerines as they are 5-(6-amino-9H-purin-9-yl) analogs of [6]-, [8]-, and [10]-gingerols, respectively using a phase trafficking-based method (Figure 26).^[71]

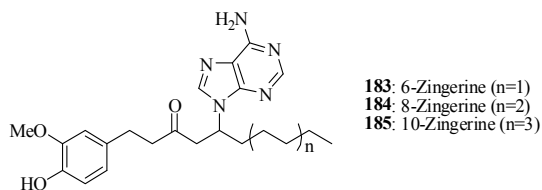
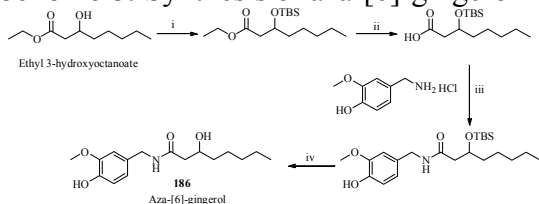


Figure 26: Isolated zingerines (**183-185**)

Synthetic Analogs

Okamoto *et al.* (2011) have synthesized aza-analog of [6]-gingerol from ethyl 3-hydroxyoctanoate as starting compound (Scheme 8).^[72] This aza- gingerol (**186**) was found to significantly reduce body weight gain, fat accumulation, and circulating levels of insulin and leptin. The mRNA levels of sterol regulatory element-binding protein 1c (SREBP-1c) and acetyl-CoA carboxylase 1 in the liver were significantly lowered in mice, fed aza- gingerol than in high-fat diet (HFD) control mice. These findings indicate that aza- gingerol enhances energy metabolism and reduces the extent of lipogenesis by downregulating SREBP-1c and its related molecules, which leads to the suppression of body fat accumulation.

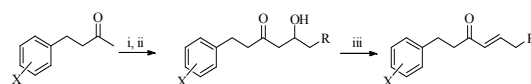
Scheme 8: Synthesis of aza-[6]-gingerol



Reagents and conditions: (i) TBSCl, imidazole, DMAP, DMF, rt, 6hr (ii) NaOH-H₂O-Methanol, rt, 3hr (iii) DCC, HOSu, DIPEA, DMAP, DMSO, DCM, rt, 48hr (iv) p-TsOH, DCM, rt, 30min

Morera *et al.* (2012) synthesized [6]-gingerol analogues and evaluated them for their transient receptor potential vanilloid (TRPV1) and transient receptor potential ankyrin type 1 (TRPA1) channel modulating properties (Scheme 9).^[73] Six potent modulators (**187-192**; Figure 27) of TRPV1 and TRPA1 have identified.

Scheme 9: Synthesis of novel [6]-gingerol analogues



Reagents and conditions: (i) LDA, THF, N₂, -78 °C, 30 min, then RCH₂CHO, THF, -78 °C, 2h. (ii) TBAF, H₂O, 0 °C, 30 min (in case of Me₃Si-protected phenol derivatives). (iii) p-TsOH, C₆H₆, reflux, 3 h.

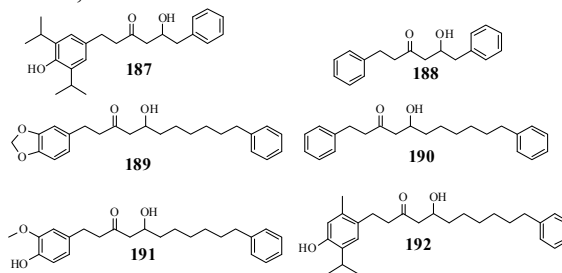


Figure 27: Structure of active modulators of TRPV1 and TRPA1

Pharmacology of Ginger

Ginger (*Z. officinale*) has been used since the ancient times as a spice and medicine in Asian, Chinese and Arabic herbal traditions. It has been used in a wide variety of diseases. The pharmacological effects of ginger are due to its bioactive chemical constituents including gingerols, shogaols, paradols, zingerones, gingerdiones and diaryl heptanoids. *Z. officinale* has been found to show many biological activities including cardiovascular effects^[74], anti-cancer effects^[75,76,77], anticoagulant effects^[78], anti-Inflammatory effects^[79], antihyperlipidemic^[80], antinociceptive effects^[81], antioxidant effects^[82], gastrointestinal effects^[83], antimicrobial activities^[84], antigenotoxic activity.^[85]

(3)
Piper nigrum
(Black pepper)

Introduction

Plants from the genus *Piper* are widely distributed throughout tropical and subtropical regions. *Piper* species have been used in traditional medicinal systems for thousands of years, including the Chinese and Indian systems, as well as in folklore medicines of Latin America and the West Indies.^[86] *Piper nigrum*, commonly known as black pepper (Figure 28), is a climbing perennial shrub. The fruit is dark green at first passing through orange-yellow to dull red when ripe. Black pepper is known to be acrid, pungent and hot. Internally, it is used as a stimulant and carminative, and induces secretion of bile. Externally, it is a rubefacient and stimulant to the skin. It is also prescribed for cholera, dyspepsia, flatulence, diarrhoea, various gastric ailments and for paralytic and arthritic disorders.^[87-90]



Figure 28: *Piper nigrum*

Chemical Constituents

Several alkaloidal and non-alkaloidal constituents from *P. nigrum* have been reported from time to time.^[91] Also, a number of naturally occurring piperidine and pyrrolidine alkamides are known to occur in *P. nigrum* and many other synthetic analogs have been synthesized, the most important being piperine, known to possess a variety of biological properties e.g. CNS stimulant, analgesic, antipyretic and antifeedant activities.^[92]

Siddiqui *et al.* (1997) isolated pipericine (193). This is N-isobutyl amide of octadeca-trans-2-cis-4-dienoic acid from the dried and crushed fruits of *P. nigrum*.^[93]

Srinivas *et al.* (1999) have isolated pyrrolidine alkamide and isopiperolein B (1-[*(E)*-10-(3,4-methylenedioxyphenyl)-dec-9-enoyl]pyrrolidine; (194; Figure 29) from the berries of *P. nigrum*.^[94]

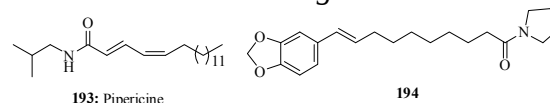


Figure 29: Structure of Pipericine (193) and Pyrrolidine alkamide (194)

Fujiwara *et al.* (2001) have isolated two new alkaloids possessing a cyclobutane ring, named pipericyclobutanamides A (195) and B (196), from the fruits of *P. nigrum* (Figure 30).^[95]

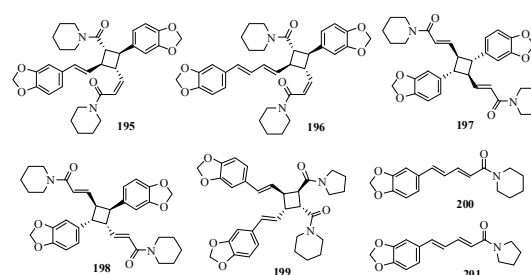


Figure 30: Structure of Pipericyclobutanamides A (195) and B (196); Dipiperamides A (197), B (198), C (199)

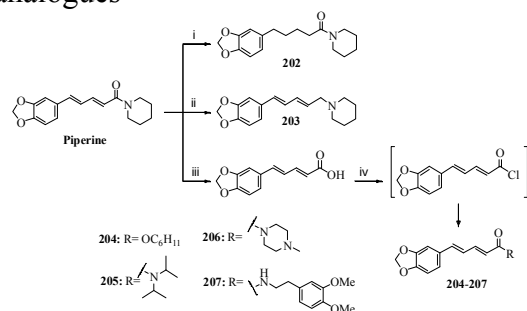
Tsakamoto *et al.* (2002) isolated dipiperamide A (197), dipiperamide B (198), and dipiperamide C (199) which are supposed to be cycloaddition products of piperine (200) and piperidine (201) (Figure 31).^[96] Cytochrome P450 (CYP) enzymes are heme containing mono-oxygenases and constitute three families, CYP1, CYP2, CYP3. The majority of these enzymes have been expressed in liver microsomes and are recognized to be responsible for drug metabolism, degradation of xenobiotics and carcinogenesis. In human liver microsomes CYP3A4 is the most abundant enzyme; approximately 30% of the total CYP was suggested to be CYP3A4. Recent investigations have shown that more than 50% of clinically used drugs are oxidized by CYP3A4. It is reported that the concomitant oral

administration of several natural products affect drug metabolism in humans by inhibiting CYP3A4. Grape fruit juice is well known example. Piperine is also known to elevate the serum level of drugs by inhibiting CYP3A4.

Synthetic analogs

Ribeiro *et al.* (2004) have synthesized derivatives of piperine (**202-207**; Scheme 10). These analogs were evaluated for their trypanocidal effects against epimastigote and amastigote forms of the protozoan parasite *Trypanosoma cruzi*, the causative agent of the incurable human disease, Chagas' disease.^[97] The activity of synthesized derivatives against Chagas' disease was found lower even than piperine itself. Standard drug used was benznidazole.

Scheme 10: Synthesis of piperine analogues



Reagents and conditions (i) EtOAc, Pd/C, H₂, 2hr (ii) DIBAL-H, toluene, -10°C, 30min (iii) KOH, ethanol, reflux, 24hr then HCl (aq), 0°C (iv) (COCl)₂, 25°C, 30min (v) DCM, alcohol or amine, 0°C, 1hr

Venkatasamy *et al.* (2004) have synthesized several piperine analogues in order to establish a structure–activity study of their ability to stimulate melanocyte proliferation (Figure 31). Results demonstrated that an aromatic ring containing at least one ether function and a carbonyl group containing side chain is essential for this activity.^[98] Three most active piperine analogues have been identified. These are 1-(3,4-methylenedioxyphenyl)-penta-2E,4E-dienoic acid methyl ester (**208**), 1-E,E-piperinoyl-isobutylamine (**209**) and 1-(3,4-

methylenedioxyphenyl)-pentanoic acid cyclohexyl amide (**210**).

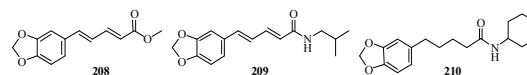


Figure 31: Structure of melanocyte proliferation activators

Kumar *et al.* (2008) have identified three piperine analogues (**211**, **212**, **213**; Figure 32), and found to be the most potent inhibitors of the NorA efflux pump.^[99] These analogues were found 2- to 4-fold more potent than piperine at a significantly lower minimal effective concentration.

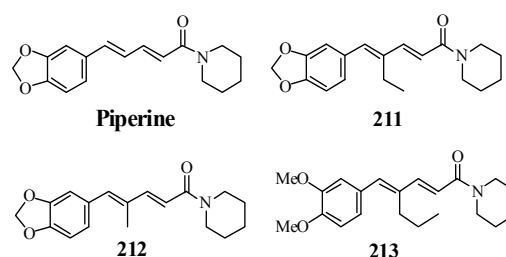
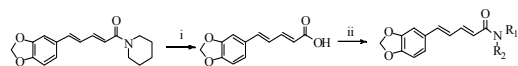


Figure 32: Chemical structure of NorA inhibitors

Bacterial multidrug efflux pumps are the major contributors of microbial resistance to several classes of antibiotics.^[100,101] By interfering with the development of resistance, clinical use of some antibiotics can be enhanced. The problem of antibiotic efflux can be overcome by addressing any of the following four strategies: (i) inhibiting drug binding to the cytoplasmic membrane pumps; (ii) inhibiting interaction of different components of a multicomponent pump; (iii) targeting energy sources of a pump; and (iv) targeting the regulatory network that controls the expression of efflux pumps.^[102] As the inhibition of an efflux pump can potentially improve the clinical efficacy of an antibiotic. Pharmaceutical companies and research institutes are therefore focusing on identifying novel efflux pump inhibitors (EPIs), which may be clinically useful.

Sangwan *et al.* (2008) have synthesized 38 piperine analogs and bioevaluated them for their *Staphylococcus aureus* NorA efflux pump inhibition (EPI) activity.^[103] Twenty-five of them were found active with potentiating activity equivalent or more than known EPIs like reserpine, carsonic acid and verapamil. The inhibitory mechanism of the compounds was confirmed by efflux inhibition assay using ethidium bromide as NorA substrate.

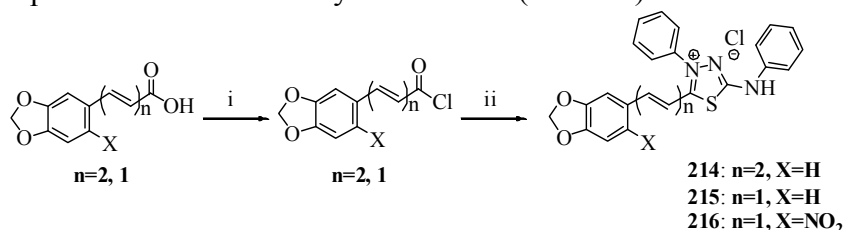
Scheme 11: Synthesis of piperine analogs as NorA efflux pump inhibitors



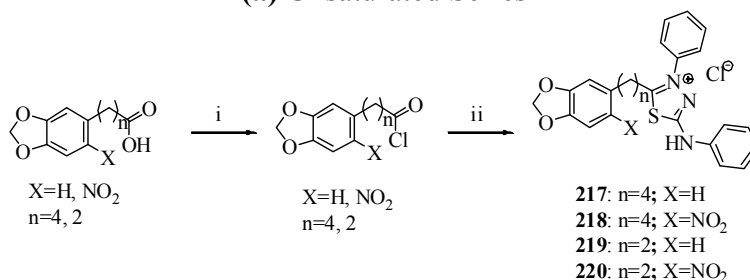
Reagents and conditions (i) Ethylene glycol, KOH, reflux, H_3O^+ (ii) SOCl_2/PhH , NHR_1R_2

Ferreira *et al.* (2008) have reported the synthesis and characterization of nine (**214-221**) new 1,3,4-thiadiazolium-2-phenylamine chlorides (mesoionic hydrochloride) derived from natural piperine.^[104] They evaluated their toxic effects against the different forms of *Trypanosoma cruzi*, and the host cell (murine macrophages). The results showed that mesoionic hydrochlorides possess the best activity profile. Therefore these compounds can be a prototype for use in the development of a new chemotherapeutic agent with high efficiency, which may be employed in the treatment of Chagas' disease. Three series were synthesized viz. unsaturated series, saturated series and aromatic aldehyde series (Scheme 12a, 12b, and 12c respectively).

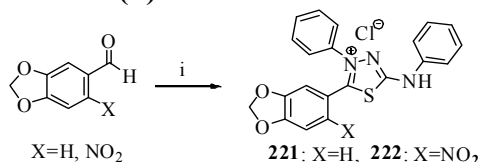
Scheme 12: Preparation of mesoionic hydrochlorides (**214-221**)



(a) Unsaturated Series



(b) Saturated Series



(c) From Aromatic aldehydes

Reagents and conditions (i) $(\text{COCl})_2$, 25°C , 2 h; (ii) 1,4 diphenylthiosemi-carbazide, 1,4-dioxane, 25°C , 24–48 h (iii) 1,4-diphenylthiosemicarbazide, TMSCl , DMF , 25°C , 24 h

Ferreira *et al.* (2011) reported leishmanicidal effects of piperine, and its derivatives (223-228), on *Leishmania amazonensis* (Figure 33).^[105]

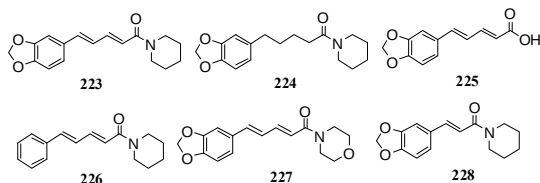


Figure 33: Chemical structure of piperine, and its derivatives

(4)

Allium sativum and *Allium cepa* (Garlic and Onion)

Introduction

Dietary supplements play a key role in the development of various human diseases, including cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes.^[106] *Allium* species such as garlic and onion (Figure 34) have been studied extensively for their health benefits. Continuous reports published in peer reviewed journals reveal the widespread interest in this class of foods. Several of the *allium* foods have been shown to reduce risks of disease or modulate metabolism to favor the prevention of diseases. Garlic, in particular, is considered to be one of the best disease-preventive foods because of its potent and widespread effects on different biochemical pathways.



Figure 34

The potency of garlic (*A. sativum*) has been acknowledged for 5000 years.

The frequent use of garlic by the Babylonians, Egyptians, Phoenicians, Vikings, Chinese, Greeks, Romans and Hindus in ancient times, has been reported.^[107] They took garlic as a remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging and many other ailments. The use of garlic as wound healing agent was very common through the middle ages into World War II.^[108] Garlic was ground or sliced and was applied directly to wounds to inhibit the spread of infections. Garlic thus acquired a reputation in the folklore system of medication of many cultures over the centuries as a therapeutic medicinal agent. To date, 3000 publications from all over the world have gradually confirmed the traditionally recognized health benefits of garlic.

Chemical constituents

Garlic contains more than 200 chemical compounds. Some of its more important ones include: volatile oil with sulphur-containing compounds: (allicin, alliin, and ajoene), and enzymes: (allinase, peroxidase and myrosinase). Allicin is responsible for its antibiotic properties and for its strong odour. Ajoene contributes to the anticoagulant action of garlic. Garlic also contains citral, geraniol, linalool, α -phellandrene and β -phellandrene. The alliin contained in garlic is also found in several members of the onion family and is considered a very valuable therapeutic compound.

Ordinarily, the vegetative parts are odour-free, and it is only during tissue damage that volatile flavour principles are generated. Interestingly, these volatile chemicals are produced through enzymatic hydrolysis of non-volatile sulfur storage compounds, termed with common name as S-alk(en)yl-L-cysteine sulfoxides (CSs). To date, four major and two minor CSs have been identified in *A. sativum*. It has received considerable attention from both

chemists and biologists alike as new sources of bioactive compounds. Early investigators identified volatile odour principles in garlic oils, however, these compounds were only generated during tissue damage and preparation. In fact, the vegetative tissues of *A. sativum* are usually odourless, and it is this observation that led to the hypothesis that the generation of volatile compounds from *Allium* species arose from non-volatile precursor substances.

Stoll and Seebrook identified stable precursor compound, (+)-S-allyl-L-cysteine sulfoxide, commonly known as alliin, for the first time in 1948.^[109] Alliin is the parental sulfur compound that is responsible for the formation of majority of the odorous volatiles produced from crushed or cut garlic. Three new sulfoxides viz. (+)-S-methyl-L-cysteine sulfoxide (methiin), (+)-S-propyl-L-cysteine sulfoxide (propiin) and (+)-S-trans-1-propenyl-L-cysteine sulfoxide or isoalliin; Figure 35) were later identified by Virtanen and Matikkala. Isoalliin (**230**) is the major sulfoxide present within intact onion tissues and is the source of the *A. cepa* lachrymatory factor.^[110,111] To date, only the L-(+)-isomers have been described in nature.^[112] Kubec *et al.* identified S-ethylcysteine sulfoxide (ethiin), not previously reported to occur in *Allium* species, as a minor component of most extracts.^[113] Horhammer *et al.* have reported S-n-butylcysteine sulfoxide in garlic (*A. sativum*).^[113,114]

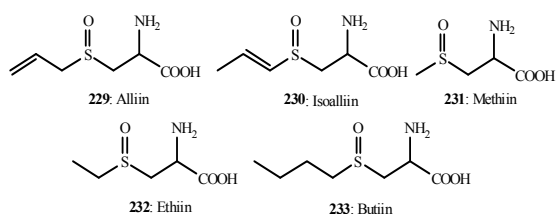


Figure 35: Compounds isolated from *Allium sativum*

Organosulfur compounds in *Allium sativum* and their transformation

Upon tissue damage the first chemical compounds to be formed are the sulfenic acids and thiosulfinates. These progenitor compounds are intermediates in the formation of the majority of sulfur volatiles.

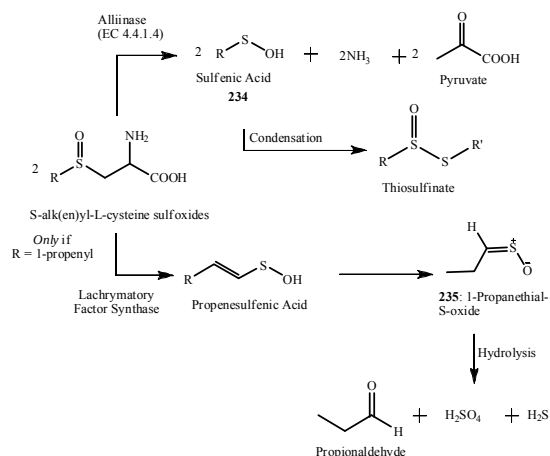
Alliinase

The enzyme alliinase [EC 4.4.1.4], a 50 kDa glycoprotein is responsible for the hydrolysis of Cysteine Sulfoxides in the presence of the cofactor pyridoxal 5'-phosphate to produce pyruvate, ammonia and sulfenic acids. In intact tissues alliinase is compartmentalised within plant vacuoles and the representative CS located in the cytoplasm.^[115]

Thiosulfinates

In freshly macerated *Allium* tissues the initial chemical compounds formed are the thiosulfinates, derived from the condensation of sulfenic acid principles.^[116] Upon tissue disruption the vacuole and cytoplasmic contents mix, promoting the enzymatic hydrolysis of the respective CS. This catalytic reaction leads to the generation of sulfenic acids that condense to form thiosulfinates. Indeed, we are aware of the presence of alliinase, catalyses the reaction leading to the generation of the lachrymatory principle found in onions, thiopropanal S-oxide (**235**)^[117] (Scheme 13).

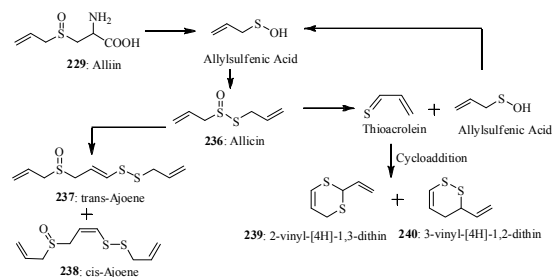
Scheme 13: Alliinase-mediated hydrolysis of S-alk(en)yl cysteine sulfoxides leads to the formation of sulfenic acids that can self-condense to form thiosulfinates, or in the case of S-trans-1-propenylcysteine sulfoxide the lachrymatory compound 1-propanethial-S-oxide.



Alliinase-mediated hydrolysis and generation of bioactive Compounds

Most flavour compounds are derived from the decomposition of thiosulfonates. For example, allicin can react to form ajoene, for which the *trans* and *cis* isomers (**237**, **238**) are recognized. In a separate pathway, allicin (**236**) can react with thiol substrates, including cysteine, to form S-allylmercapto-L-cysteine. Alternatively, allicin can further decompose to form allyl sulfenic acid and thioacrolein. Two molecules of allyl sulfenic acid can condense to re-form one molecule of allicin or alternatively, two molecules of thioacrolein. Thioacrolein is highly reactive and can undergo self-condensation by a Diels–Alder reaction to generate the cyclics 2-vinyl-[4H]-1,3-dithin (**239**) and 3-vinyl-[4H]-1,2-dithin (**240**, Scheme 14).^[118]

Scheme 14: Generation of garlic flavour components



Allyl sulfides

Ajoene

Ajoene is a stable disulfide containing natural product formed by the combination of two molecules of allicin (Figure 36). A mixture of two stereoisomers that differ in the configuration of the central double bond, ajoene has a wealth of different biological activities, including antithrombotic, antitumor, antifungal and antiparasitic effects.^[119] Its interaction with human glutathione reductase is well characterized.^[120] The co-crystal structure shows a disulfide bridge that connects an enzyme's active site residue Cys58 with the sulfoxide containing half of ajoene via a disulfide bridge. Ajoene increases PKCδ-dependent Nrf2 activation, GCL induction, and the cellular GSH concentration, which may contribute to protecting cells from oxidative stress.^[121]

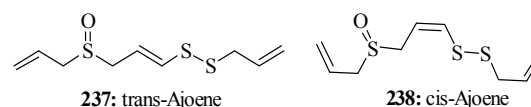


Figure 36: Structure of ajoenes

Allyl sulfides (diallyl sulfide, diallyl disulfide, and diallyl trisulfide) are lipophilic thioesters derived from alliin. They show differential upregulation on the protein or gene expression of phase II detoxifying enzymes with strength in the order of diallyl trisulfide, diallyl disulfide, diallyl sulfide (**239–241**, Figure 37),^[122] however, some reports suggest that allylsulfide causes a striking increase in the greatest number of genes.^[123] High intake of raw or cooked garlic have protective effects against stomach and colorectal cancers in a site-specific case-control study.^[124]

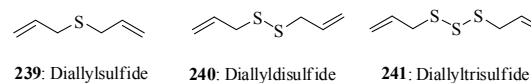


Figure 37: Structure of allyl sulfides

Some unusual sulfur compounds

A second group of very unusual sulfur compounds that are likely to be derived from bis- α - β -unsaturated thiosulfinates has also recently been identified in *Allium* extracts and shown to be biologically active. Two isomers of 2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide were identified in onion extracts.^[125-127] These compounds are trivially named *cis*- and *trans*-zwiebelanes (**242**, **243**; Figure 38). Recently, research has focused on determination of the mechanisms of formation of these novel compounds, the chemistry of which is highly complex. Moreover, a structurally similar (Z,Z)-($\pm$)-2,3-dimethylbutanedithial S,S'-dioxide (**244**) has been identified in onion extracts using NMR methodologies and subsequently extracted (Figure 38). (Z,Z)-($\pm$)-2,3-dimethylbutanedithial S,S'-dioxide is the first example of a bis(thial S-oxide).^[128,129]

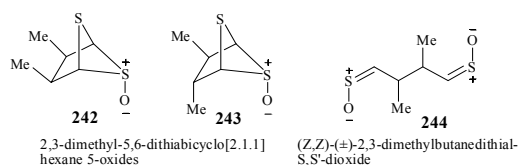
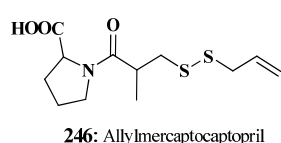


Figure 38: Unusual sulfur compounds isolated from onion

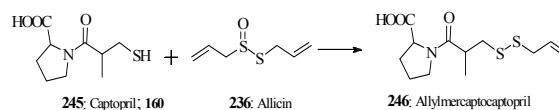
Allylmercaptocaptopril –A potent antihypertensive drug

A novel antihypertensive drug was synthesized through the reaction of the pharmaceutical drug Captopril (**246**, Figure 39) with the *Allium*-derived thiosulfinate allicin (Scheme 15).^[130] Captopril possesses potent antihypertensive properties due to its ability to inhibit the angiotensin-converting enzyme. Similarly, allicin also inhibits hypertension by reducing serum cholesterol and triglyceride levels. Therefore, the authors proposed that a



combination of both the drugs with two different pharmacological sites of action, may provide better protection against hypertension. The reaction product thus obtained by the reaction between captopril and allicin was given the name allylmercaptocaptopril. It is a nonsymmetric disulfide that combines the specific drug activity of captopril and the beneficial properties of allicin.

Scheme 15: Synthesis of allylmercaptocaptopril



Recently Rai *et al.* synthesized seven new derivatives of diallyldisulfide (Scheme 16) and evaluated their DNA binding, and cytotoxic activities.^[131] These analogs were found less toxic and equally effective as the statins. Out of which compound **247** and **248** were found most active.

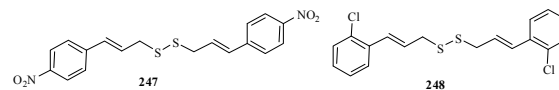
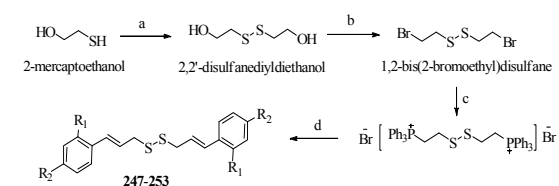


Figure 40: Active analogues

Scheme 16: Synthesis of diallyldisulfide



Reagents and conditions (a) Br_2 , KHCO_3 , DCM (49%); (b) HBr , H_2SO_4 (59%); (c) PPh_3 , DMF, reflux (72%); (d) NaOEt , substituted benzaldehyde (70–79%).

Table 1: Synthesized compounds

Compound d	247	248	249	250	251	252	253
R_1	H	Cl	Cl	Br	Br	H	H
R_2	NO ₂	H	Cl	H	Br	F	CF ₃

Pharmacology

Al-Qattan *et al.*^[132] reported the antihypertensive effect of aqueous garlic extract. Garlic oil exerts its effects by modulating lipid peroxidation and enhancing antioxidant and detoxifying enzyme systems.^[133] Garlic and onion-juices were found to reduce the plasma glucose levels by 70% and 68%, respectively compared with the alloxan induced diabetic group in rats.^[134] Most recently allicin was found to inhibit the epithelial sodium channel (ENaC) which is a key factor in the trans-epithelial movement of sodium, and consequently salt and water homeostasis in various organs. Dysregulated activity of ENaC is associated with human diseases such as hypertension, the salt-wasting syndrome pseudohypoaldosteronism type 1, cystic fibrosis, pulmonary oedema or intestinal disorders.^[135]

Weiss *et al.* reported that aged garlic extract helps in restoring effect of nitric oxide bioavailability in cultured human endothelial cells and prevention of tetra- hydrobiopterin oxidation. Aged garlic extract (AGE) is a garlic preparation rich in water-soluble organosulfur compounds, such as S-allylcysteine and S-allylmercaptocysteine. This suggests that aged garlic extract might be useful in the prevention of endothelial dysfunction.^[136]

(5) *Crocus sativus* (Saffron or Kesar)

Introduction

Crocus sativus L. belonging to the family Iridaceae (syn-kesar, Saffron). It comprises the dried red stigma and is widely cultivated in Iran and other countries such as India and Greece. Since

ancient times, saffron, the dried stigma of the plant *C. sativus L.* has been extensively used as a spice and food colorant. It has been reputed to be efficacious for the alleviation and treatment of ailments in traditional medicine. In addition to the three major chemical constituents including crocin, picrocrocin and safranal,



saffron contains more than 150 volatile and aroma-yielding compounds including terpenes, terpenols, their esters, carotenoids, carbohydrates, proteins, anthocyanins, vitamins and minerals. The bitter taste and peculiar hay-like fragrances are caused by chemicals picrocrocin and safranal.

Chemical constituents

The principal active substances present in saffron are aglycon (without glycon unit) crocetin (**254**), crocin (**255**), picrocrocin (**256**) and safranal (**257**, Figure 41). Other homoanalogues of crocin have also been isolated (Figure 42). Safranal is a cyclic terpenic aldehyde and picrocrocin is a glycoside combining with glucose and an aglycon, 4-hydroxy- β -cycloctral. Crocin is a crocetin digentiobiose ester whereas crocetin (the aglycon of crocin) is 8,8'-diapocarotenedioic acid.^[137] Crocetin, a diterpenic compound, has been identified as the most potent carotenoid responsible for different pharmacological properties in saffron.^[137]

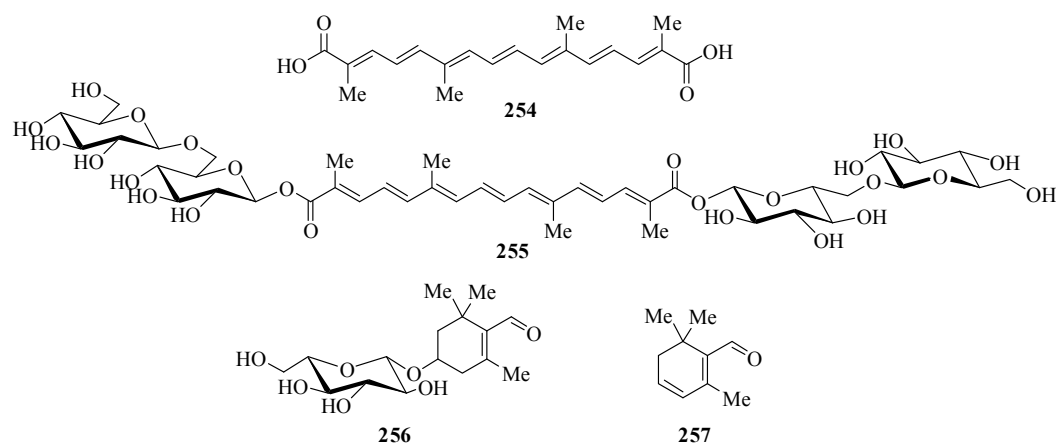


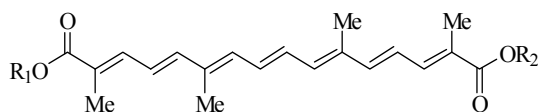
Figure 41: Chemical constituents of Saffron (*C. sativus*) Crocetin (**254**); Crocin (**255**); Picrocrocin (**256**); Safranal (**257**)

Crocetin

Crocetin belongs to the large family of natural dyes known as carotenoids, but it does not have a pro-vitamin function. The constituents of this class of small molecule compounds are mostly polyunsaturated hydrocarbons (the formula is $C_{40}H_{56}$) or their oxygenated derivatives. There are small groups of carotenoids that are carboxylic acids. Among those crocetin (the aglycan of crocin), 8,8'-Diapocarotenedioic acid, characterized by a diterpenic and symmetrical structure with alternating double bonds and four methyl groups.

Crocin

Main secondary metabolites of saffron are a homologous series of carotenoid-glycosyl esters of C20-dicarboxylic acids. The so called crocins (Figure 42) account for up to 30% and are responsible for the intense yellow color of saffron.^[138,139]



- 255:** Crocin-1; R1= R2 = gentiobiosyl-
- 258:** Crocin-2; R1= gentiobiosyl-, R2 = glucosyl-
- 259:** Crocin-3; R1= R2 = glucosyl-
- 260:** Crocin-4; R1= gentiobiosyl-, R2 = H
- 261:** Crocin-5; R1= glucosyl-, R2 = H

Figure 42: Crocin homo analogues isolated from *C. sativus*

Pharmacology

C. sativus L., is used in folk medicine as an antispasmodic, eupeptic, gingival sedative, anticatarrhal, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac and emmenagogue. Furthermore, modern pharmacological studies have demonstrated that saffron extract or its active constituents have antitumor effects, radical scavenging properties, anti-hyperlipidemic effects. Of the carotenoids present in saffron, highly water-soluble crocin (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin) is responsible for the majority of its color, and appears to possess various health-promoting properties, as an antihypertensive, anticonvulsant, antitussive, antigenotoxic and cytotoxic effects, anxiolytic aphrodisiac, antioxidant, antidepressant, antinociceptive, anti-inflammatory, antioxidant, antitumor, memory enhancer, relaxant activity and increases blood flow in retina and choroid. In addition, saffron has found to have better efficacy profile along with no major toxicity in experimental models.

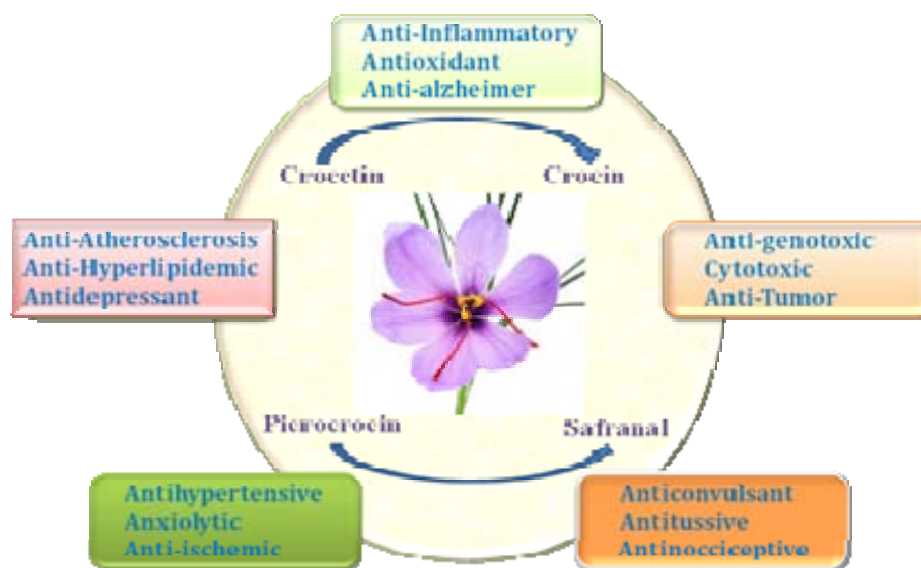


Figure 43: Pharmacology of *C. sativus* (Saffron)

Crocetin and cancer

Saffron and its derivatives particularly crocetin have demonstrated significant anticancer activity in breast, lung, pancreatic and leukemic cells. **Table 2** summarizes the effects of crocetin against several cancer types and also presents the underlying mechanisms of

action. Crocetin was found to cause stabilization of β -amyloid ($A\beta$) oligomers and prevented their conversion into $A\beta$ fibrils by inhibiting $A\beta$ fibril formation and destabilizing pre-formed $A\beta$ fibrils.¹⁶⁰

Table 2: *In vitro* and *In vivo* Effects of Crocetin against Several Cancers

Types of Cancers	Cell lines/Animal Models	Factors Affected	References
Breast Cancer	MCF-7, MDA-MB-231	↓Proliferation	140
	MCF-7, MDA-MB-231	↑Apoptosis	141
Cervical Cancer	HeLa Cells	↓DNA, RNA and protein synthesis	Abdullaev & Frenkel 142
	HeLa Cells	↑Apoptosis	Tavakkol-Afshari <i>et al.</i> 143
	HeLa Cells	↓RNA polymerase activity	Abdullaev 144
	HeLa Cells	↑tRNA interaction	Kanakis <i>et al.</i> 145
	HeLa Cells	↓RNA, DNA and protein synthesis	Escribano <i>et al.</i> 146
Colorectal Cancer	HCT-116, SW-480, and HT-29	↓Proliferation	Aung <i>et al.</i> 147
Leukemia	HL60	↑Cytotoxicity and ↓proliferation	Tarantilis <i>et al.</i> 148
	L1210 and P388	↑Cytotoxicity and ↓proliferation	Morjani <i>et al.</i> 149

	K562	↑Cytotoxicity and ↓proliferation	Tarantilis <i>et al.</i> 148
Liver Cancer	Wistar rat (AFB1)		Morjani <i>et al.</i> 149
	C3H1OT1/2 cells	↓Lipid peroxidation	Wang <i>et al.</i> 150
	Wistar rat (AFB1)	↓Reactive oxygen species	Wang <i>et al.</i> 151
	C3H1OT1/2 cells	↓ DNA-adduct formation	Chang <i>et al.</i> 152
	Wistar rat (AFB1)		
	C3H1OT1/2 cells		
	HepG2	↓Proliferation, ↑Apoptosis	Tavakkol-Afshari <i>et al.</i> 153
Lung Cancer	Swiss albino mice (B[a]P)	↓Lipid peroxidation, ↑GST, ↑catalases, ↑superoxide dismutase	Magesh <i>et al.</i> 154
	Swiss albino mice (B[a]P)	↓Polyamine	Magesh <i>et al.</i> 155
	A549 lung carcinoma	↓DNA, RNA and protein, ↓RNA polymerase II	Abdullev1994 156
Pancreatic Cancer	VA-13 fetal lung fibroblast	↓DNA, RNA and protein, ↓RNA polymerase II	Abdullev1994 156
	MIA-PaCa-2, BxPc3, Capan-1 and ASPC-1 cells;	↓Proliferation,	Dhar <i>et al.</i> 157
	Athymic mice xenograft model (MIA-PaCa-2)	↑Cdc2 phosphorylation, ↓Cdc25c, ↓cyclin B1,	
		↓ EGFR phsophoryaltion,	
		↑apoptosis, ↑Bax, ↓Bcl-2,	
Skin Cancer		↓Tumor formation	
	Swiss Webster mice (DMBA and croton oil)	↓Tumor formation	Gainer <i>et al.</i> 158; Mathews-Roth 159

(6)

***Trigonella foenum-graecum*
(Fenugreek or Methi)****Introduction**

Fenugreek (*Trigonella foenum-graecum*; (Figure 44) is one of the oldest medicinal plants. This plant is thought to originate from India or the Middle East. It is now primarily grown in India and in the Mediterranean countries. It is an annual herbaceous plant belonging to the Fabaceae family, 40-60 cm height, with alternate trifoliate leaves and pale yellow flowers. The common names of the plant are methi, Greek hayseed, and bird's foot. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use.



Figure 44: *T. foenum-graecum* L.: Plant and Seeds

Chemical Constituents

T. foenum-graecum has been extensively explored for its chemical constitution owing to its profound antihyperglycemic and antidyslipidemic activities. It contains steroids, Nitrogenous compounds, polyphenolic substances, volatile constituents, amino acids, etc.^[161] Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans), 20-30% proteins high in lysine and tryptophan, 5-10% fixed oils

(lipids), pyridine alkaloids, mainly trigonelline (**262**) (0.2-0.36%), choline (0.5%), gentianine and carpaine, the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin, free amino acids, such as 4-hydroxyisoleucine (**263**; 0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6-1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin (**264**), yamogenin, tigogenin, neotigogenin), cholesterol and sitosterol, vitamins A, B1,

C and nicotinic acid and 0.015% volatile oils (nalkanes and sesquiterpenes).^[162,163] Anis *et al.*^[164] have reported the presence of three steroidal sapogenins i.e. diosgenin, gitogenin and tigogenin (Figure 45). The use of more sophisticated analytical techniques including coupled GC-MS have detected and identified ten different sapogenins.^[165] Presence of a sapogenin peptide ester, fenugreekine (**265**; Figure 45) has been reported.^[166]

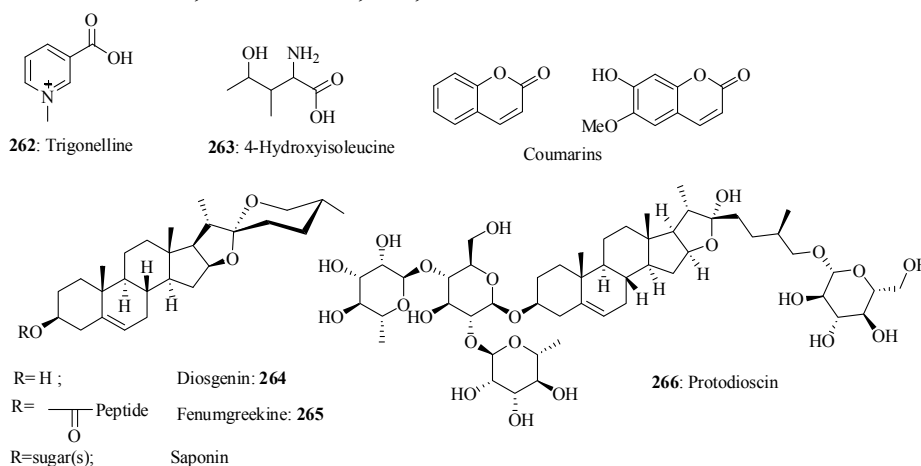


Figure 45: Antidiabetic or hypocholesterolaemic compounds in fenugreek seeds.

Trigonelline (**262**) (Figure 45) is an important alkaloidal component of the seeds.^[167] Diosgenin and trigonelline present in the seed and leaves of this legume plant contribute to anti-diabetic and hypocholesterolaemic properties attributed to the plant. Fenugreek, like other legumes, is rich in arginine, alanine and glycine; but poor in lysine content.^[168] However, 4-hydroxyisoleucine (Figure 45) has been found to be a major free amino acid in the seeds.^[169] The aromatic constituents of the seeds have been elucidated^[170] and include *n*-alkanes, sesquiterpenes and some oxygenated compounds such as hexanol and γ -nonalactone. The seeds are also known to contain flavonoids, carotenoids, coumarins and other components^[171] with a very low LD₅₀. A novel antidiabetic compound containing fraction was discovered from fenugreek seeds and found to contain approximately 30% protodioscin (**266**;

Figure 45) as one of the active principles.^[172]

Pharmacology

Fenugreek seeds have been known for a long time for their antidiabetic action. Several groups^[173] have been reported anti-hyperglycemic activity in the alcoholic and aqueous extracts of *T. foenum-graecum*. Fourier^[174] observed that the administration of coarsely ground fenugreek seeds improved severe diabetes in human subjects. Ghafghazi *et al.*^[175] have shown that an extract of fenugreek prevented the hyperglycaemia induced by cadmium and alloxan in rats. The aqueous extract of its leaves given both orally and intraperitoneally possessed a hypoglycemic effect in normoglycemic and alloxan induced hyperglycemic rats.^[176]

In humans, fenugreek seeds exert hypoglycemic effect by stimulating glucose dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of α -amylase and sucrose.^[177] Fenugreek seeds also lower serum triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). These effects may be due to saponins, which increase biliary cholesterol excretion in liver, leading to lowered serum cholesterol levels.^[178] Amin *et al.*^[179] showed strong antidiabetic effect *in vivo*.

4-Hydroxyisoleucine (4-HIL) is an active principle from fenugreek seeds

Fenugreek is known for its use in traditional herbal medicine and is known to have hypoglycaemic and hypolipidemic properties. Hypoglycaemic effects of fenugreek have been attributed to alkaloid components and to the modified amino acid 4-hydroxyisoleucine (4-HIL),^[180] which is present in Fenugreek seeds as the 2R, 3S, 4R form, out of eight possible stereoisomers.^[181]

Fowden^[182] had isolated this unusual amino acid i.e., 4-hydroxyisoleucine (4-HIL) and identified it for the first time from the seeds. 4-HIL increases glucose-induced insulin release, in the concentration range of 100 μ M to 1 mM, through a direct effect on isolated islets of langerhans from both rats and humans.^[180] Moreover, 4-HIL did not interact with other agonists of insulin secretion (leucine, arginine, tolbutamide and glyceraldehyde). Therefore, 4-HIL insulinotropic activity might, at least in part, account for antidiabetic properties of fenugreek seeds. This secretagogue might be considered as a novel drug with potential therapeutic use for the treatment of NIDDM.^[183] Our recent studies indicated that the 4-hydroxyisoleucine exerts antidiabetic activity in hamster model.^[184]

Conclusion

This review covers the literature mainly from 2008 to December 2013. In this review we have compiled reports on isolation of new phytochemicals and chemical transformations of key constituents from dietary spices to obtain more potent derivatives with better pharmacological profile. This review also focused on the use of isolated natural products from dietary spices and their synthetic derivatives and analogues for different diseases. Recent statistical data regarding global and Indian nutraceutical market have also been presented.

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