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2.1. Medicinal plants uses in health care: An introduction

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, plant products were the main source of drugs (De Pasquale, 1984). Plants have formed the basis of traditional systems of medicine and continue to provide mankind with new remedies. Plant products as part of food or botanical portions and powder have been used with varying success to cure and prevent diseases. It has been reported that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after ethnomedical evaluation (Eloff, 1998; Raskin et al., 2002; Gurib-Fakim, 2006).

Ethnomedicinal plants are used both for primary health care and for treating chronic diseases such as AIDS, cancer, hepatitis disorders, heart and old age related diseases like memory loss, osteoporosis and diabetic wound. In the Indian coded system (Ayurveda, Unani, Siddha, Amchi), Ayurveda currently utilizes as many as 1000 plants and over 8000 formulations of recognized merit. Similarly, 600-700 plants are utilized by other systems like Unani, Siddha and Amchi (Krishna, 2003). Ayurveda is perhaps the most ancient of all traditional systems of medicine, probably older than the Chinese medicine. It is considered to be the origin of systemized medicine. The first record written on clay tablets in cuneiform is from Mesopotamia and date from about 2600 B.C. The history of medicinal plants date back to Rigveda is perhaps the oldest repository of human knowledge which was written around 4500-1600 B.C. The Ayurveda, developed around 2500 B.C. described the detail account of many drugs, which are even in use today. Ancient Ayurveda includes the comprehensive work of Chraka (1000 B.C.) and Sushruta (800 B.C.) and provide the base for the Materia Medica. Hippocrates (in the late fifth century B.C.) mentioned 300-400 medicinal plants (Schultes, 1978). In the first century A.D., Dioscorides wrote Demateria medica, a medicinal plant catalogue which became the prototype for modern pharmacopias. The Bible offers description of approx. 30 healing plants. The Mohammedan culture enriched the vegetable Materia medica, which was further improved by those in Greece, Arabia and Persia (Arora, 1965).

Hakim Ibn Sina (981-1037 C.E.) known as Avicena in the West laid down the
foundation of the Greco-Arab system of herbal medicine (Unani, Tibb), based on the philosophy of individualized treatment considering the variation amongst the individuals similar to the concept of pharmacogenetics in conventional medicine. This system of medicine has found its root in India and become well established system of Indian medicine (Gilani and Atta-ur-Rahman, 2005). The biogeographic position of India is unique and has diverse ecosystem, ranging from the humid tropics of Western Ghats to the Alpine zone of Himalaya and from the dry deserts of Rajasthan to the tidal mangroves of the Sunderbans and hence India is endowed with a rich diverse flora (Dahanukar and Hazra, 1995).

With the realization that ethnomedicinal plants are a repository of numerous potential medicines, concerted efforts from India, China and other countries around the globe were made to evaluate these plants scientifically for various biological and therapeutic properties and alternative source of drugs. The traditional systems of medicine have now been recognized and accepted as alternative/complementary system of medicine for primary health care and for some chronic diseases (Cowan, 1999).

Although the first chemical substance to be isolated from plants was benzoic acid in 1560, the search for useful drugs of known structure did not begin until 1804 when morphine was separated from *Papaver somniferum* L. (Opium). Since then several drugs from higher plants have been discovered but less than 100 of defined structure are of common use today. About 55 drugs are widely employed in Western medicine. Drugs like aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, resperine, taxol, tubocurarine, vincristine, and vinblastine are few examples of what medicinal plants given us in the past (Raskin et al., 2002, Tiwari, 2004; Ahmad et al., 2006).

Plants have interdependent pathways that lead to the synthesis of numerous metabolites. Some of these metabolites are physiologically active and are being exploited for human and animal use, as they are being succeeded for various therapeutic properties and as source of new drugs. Recent findings showed their useful properties like anticancer, antitumour, antimutagenic, antioxidant, hepatoprotective, antiviral, antimalarial, antidiysenteric, antiseptic, antistress and immunotherapeutic, antibacterial, antifungal and several other pharmacological
actions (Cowan, 1999; Tiwari, 2004; Musarrat et al., 2006; Nostro, 2006; Kaefer and Milner, 2008; Heinrich, 2010).

2.2. Drug resistance in bacteria: A global problem

Resistance mechanisms allow bacteria to survive in the presence of adverse conditions that can result from acquired or intrinsic cell changes. Bacteria may be intrinsically resistant to antimicrobial drugs, or may acquire resistance by de novo mutations or via the acquisition of resistance genes from other microorganisms (Fajardo et al., 2008). Acquisition of new genetic material by antimicrobial susceptible bacteria from those resistant counterparts may occur through gene transfer mechanisms such as conjugation, transformation, and transduction (Hurdle et al., 2005; Tenover, 2006; Alekshun and Levy, 2007). Thus, these acquired resistance genes may enable a bacterium to produce enzymes that inactivate the antibacterial product, to modify the target site, to produce an alternative metabolic pathway(s) that bypasses the action of the antibacterial product, or to express efflux mechanisms that prevent the antibacterial from reaching its intracellular target (Spratt, 1994; Webber and Piddock, 2003; Woodford and Ellington, 2007).

Efflux mechanisms, both drug-specific and multidrug, are important determinants of intrinsic and/or acquired resistance to these antimicrobials (Lomovskaya and Watkins, 2001). Intrinsic resistance to antimicrobials is a natural property of bacteria. This is frequently associated with cellular impermeability imparted by the outer layers, limiting the uptake of antimicrobial products (Russell, 2001). It is widely recognized that Gram-negative bacteria are generally less susceptible to antimicrobial products than Gram-positive; their cell walls present a more significant barrier to entry (White et al., 2004). The presence of efflux systems coupled with the narrow porin channels in the outer membrane which restricts diffusion of antimicrobials into the cells is responsible for the very high intrinsic resistance of Gram-negative bacteria (McDonnell and Russell, 1999). In addition to the impaired uptake, some bacteria demonstrated intrinsic resistance through the inactivation and biodegradation of antimicrobial products by natural evolutionary mutations leading to modifications in protein configuration (Nishihara et al., 2000; Dantas et al., 2008). In addition, indiscriminate use of antibiotic and non medical uses has resulted in development of
multidrug resistance in pathogenic bacteria. Most commonly encountered multiple resistant isolates include strains of *Staphylococcus aureus*, resistant to methicillin and other unrelated antibiotics and more popularly known as MRSA (Sakagami, 2006).

Similarly among Gram negative pathogens, members of enterobacteriaceae are common producers of β-lactamases and are resistant to β-lactam antibiotics. A recent group of extended spectrum β-lactamases (ESβL) producer bacteria showing resistance to new generations of 4-quinolone drugs is considered as emerging global threat. These ESβL producing bacteria harbour conjugative plasmid which might play a critical role in dissemination of such resistance genes among other sensitive or less resistant bacteria. Multidrug resistance has become more problematic in several other pathogenic bacteria such as *Mycobacterium tuberculosis*, *Haemophilus influenzae*, *Streptococcus pneumoniae* etc. However, frequency of occurrence in *S. aureus* and enteric bacteria are more and constitute a major reservoir for spread of plasmid born resistance in many other pathogenic bacteria through genetic exchange mechanisms (Aqil et al., 2006; Bisht et al., 2006; Livermore, 2007).

2.2.1. Antibacterial activity of medicinal plants against drug resistant bacteria

Antibacterial activity of medicinal plant extracts and several phytocompounds are known since long. However, it has only been used either in traditional medicine/primary health care or as marker for testing compounds activity in natural chemistry. In recent times, due to increased trend of multidrug resistance among pathogenic bacteria or diminishing trend of new antibacterial drug discovery, the role of natural products including medicinal plants have received attention among academic and research institutions for search of novel compounds against drug resistant bacteria (Ahmad et al., 2008).

There is a great structural diversity exist among antimicrobial phytocompounds (Cowan, 1999). Major groups of phytocompounds include essential oils and isolated compounds such as alkaloids, flavonoids, sesquiterpenes, lactones, diterpenes, triterpenes or naphthoquinones etc (Rios and Recio, 2005). Considerable amount of work have been published on antimicrobial activity of medicinal plants from different parts of the world including India, Pakistan, Iran, China, Brazil, Japan, Thailand, USA
and in many countries (Bhakuni et al., 1988; Bhakuni et al., 1990; Aswal et al., 1996; Ahmad et al., 1998; Cowan, 1999; Dorman and Deans, 2000; Aqil and Ahmad, 2003; Bonjar, 2004; Uzun et al., 2004; Durate et al., 2005; Rios and Recio, 2005; Kumar et al., 2006).

Most of the studies are directed to see the activity against a variety of test bacteria including both pathogenic and non-pathogenic strains. However, active compounds for antibacterial activity are yet to be determined in most of the cases. Many workers have also made specific studies on therapeutic potential of medicinal plants against pathogenic bacteria (O’Gara et al., 2000; Ahmad and Beg, 2001; Voravuthikunchai et al., 2004; Eloff and McGaw, 2006). Similarly, several workers have reported targeted screening against multidrug resistant bacteria such as methicillin resistant Staphylococcus aureus (MRSA), vancomycin resistant enterococci (VRE), and Mycobacterium tuberculosis etc (Rios and Recio, 2005; Nostro, 2006; Sakagami, 2006; Aqil and Ahmad, 2007).

However since last decade the efforts made by various scientists on screening and search for antibacterial extracts are more focussed against drug resistant bacteria. It is hoped that if multidrug resistant bacteria are sensitive to plant extracts or phytocompounds it might be having active compounds with therapeutic significance in combating drug resistant bacteria alone or in combination with antibiotics. This review mainly focuses on studies conducted on medicinal plants for their activity against drug resistant bacteria in different parts of the world.

The effects of an aqueous extract of garlic and its active constituent allicin were tested against 40 drug resistant isolates of the strains of Shigella dysenteriae type 1 and Shigella flexneri, enterotoxigenic Escherichia coli and Vibrio cholerae. The aqueous extract and allicin were shown to have potentially significant activity against all of the bacteria tested, while out of five standard antibiotics, only gentamicin was active. Both allicin and the aqueous extract had a broad spectrum activity as antibacterial agents. Allicin appeared to have the strongest activity (Ahsan et al., 1996). In the same year, ajoene, garlic derived sulphur containing compound had been isolated that prevent platelet aggregation, exhibited broad spectrum antimicrobial activity. Growth of Staphylococcus aureus and Lactobacillus plantarum were inhibited by <20 μg/ml
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(Naganawa et al., 1996). Similarly, anti-MRSA activity of Sofora flavonone G (SFG) and synergy between SFG and antibacterial agents against MRSA were demonstrated. The MICs of SFG against 27 strains of MRSA were found in the range of 3.13 to 6.25 µg/ml (Sakagami et al., 1998).

Tea tree oil showed a rapid decrease in the survival of different pathogenic drug resistant bacteria including Enterococcus faecalis, methicillin resistant S. aureus, and Pseudomonas aeruginosa in the first 2 h of incubation. The oil killed the MSSA strains within the 30 min while two MRSA strains were killed in 1.5 h and 3.5 h. However, some of the Pseudomonas isolates showed 99% decrease in the viable count after 1 h of incubation (May et al., 2000).

Nostro et al. (2001) demonstrated the effect of Helichrysum italicum on growth and enzymatic activity of S. aureus. H. italicum extract had an inhibitory effect on S. aureus strains reducing both their growth and some of the enzymes such as coagulase, DNAse, thermonuclease and lipase. Helichrysum italicum extract thus could be a novel antimicrobial agent, less toxic to human skin and tissues, worthy of further studies.

Ahmad and Beg (2001) screened 45 Indian medicinal plants against drug resistant Gram positive and Gram negative bacteria. Of these 40 extracts showed varied level of activity while 12 plants viz. L. inermis, Eucalyptus sp., H. antidysentrica, H. indicus, C. equistifolia, T. beberica, T. chebula, E. officinalis, C. sinensis, S. aromaticum and P. granatum exhibited broad spectrum activity. Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins, alkaloids and saponins and flavonoids as major active constituents.

Kumarasamy et al. (2002) evaluated hexane, dichloromethane and methanol seeds extracts of 21 Scottish plant species from 14 different families for antibacterial activity against 11 pathogenic bacteria. Methanol extracts of 11 plant species showed significant antibacterial activity. Malva moschata and Prunus padus were active against five bacterial species, Reseda lutea against four, Centaurium erythraea and
*Crithmum maritimum* against three, *Calluna vulgaris* against two, and *Armeria maritima, Centaurea scabiosa, Daucus carota, Rosa canina* and *Stellaria holostea* against one bacterial species. *C. erythraea* and *P. padus* were also active against methicillin resistant *Staphylococcus aureus*.

Four compounds were isolated from leaves of guava (*Psidium guajava* L.) and identified as flavonoid glycosides, morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside, and two known flavonoids, guaijavarin and quercetin. The MIC of morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside were 200 μg/ml for each against *Salmonella enteritidis*, and 250 μg/ml and 300 μg/ml against *Bacillus cereus*, respectively (Arima and Danno, 2002). Similarly, calozejyloxanthone, a compound from an endemic species of Sri Lanka, *Calophyllum monii* was obtained and found to be active against vancomycin resistant enterococci (VRE) and vancomycin sensitive enterococci (VSE) with MIC values of 6.25 μg/ml and 12.5 μg/ml respectively (Sakagami *et al.*, 2002).

Extracts of fourteen traditional used Brazilian medicinal plants used to treat infectious diseases were assessed for potential antibacterial activity against MDR *S. aureus* strains. These strains were susceptible to extracts of *Punica granatum* and *Tabebuia avellanedae*. A mixture of ellagitannins isolated from *P. granatum* and two naphthoquinones isolated from *T. avellanedae* demonstrated antibacterial activity against all *S. aureus* strains tested (Machado *et al.*, 2003).

Antibacterial activity of tea is well known and has been demonstrated by many workers throughout the world. Epicatechin gallate and epigallocatechin gallate, two major flavonoids found in tea leaves, inhibited antibiotic efflux pumps in methicillin resistant *S. aureus* (Gibbons *et al.*, 2004); as well as potentiated the antibacterial activity of β-lactam antibiotics against MDR strains of *S. aureus* (Hu *et al.*, 2002; Zhao *et al.*, 2002).

Evaluation of seven ethanolic extracts and three aqueous extracts from various parts (leaves, stems and flowers) of *Acacia aroma* against 163 strains of multiple antibiotic resistant bacteria showed activity against Gram-positive bacteria. However, only leaf and flower extracts showed activity against Gram-negative bacteria (Arias *et al.*, 2002).
Similarly, Reddy et al. (2004) carried out the bioactivity guided fractionation of the petroleum ether extract of the berries of *Piper nigrum* afforded 2E, 4E, 8Z-N-isobutyleicosatrienamide, pellitorine, trachyone, pergumidiene and isopiperolein B. Pergumidiene and trachyone are isolated for the first time from *P. nigrum*. Isolated compounds were active against *Bacillus subtilis, B. sphaericus, S. aureus, Klebsiella aerogenes* and *Chromobacterium violaceum* respectively.

Oregano essential oil, carvacrol and thymol were found efficacious against 26 MSSA and 21 MRSA using agar dilution methods. Carvacrol and thymol showed no significant difference in activity against MSSA and MRSA (Nostro et al., 2004). In another study, an isoflavone from the roots of *Erythrina variegata* (Leguminosae) characterized as 2, 4-dihydroxy-8-γ-γ-dimethyl allyl 2″ 2″-dimethyl pyrano [5″, 6″: 6, 7] isoflavone (bidwillon B) inhibited the growth of 12 MRSA strains with minimum inhibitory concentrations of 3.13 to 6.25 μg/ml, while MICs of mupirocin were 0.20-3.13 μg/ml. Mupirocin is a naturally occurring agent produced by *Pseudomonas fluorescens* and has been successfully used to reduce substantially the nasal and hand carriage of MRSA (Sato et al., 2004).

Akinyemi et al. (2005) tested six medicinal plants used in South-West Nigerian unorthodox medicine for anti-MRSA activity. Both water and ethanol extracts of *Terminalia avicennioides, Phylantus discoideus, Ocimum gratissimum* and *Acalypha wilkesiana* were effective on MRSA. The MIC and MBC of these plants ranged from 18.2 to 24.0 μg/ml and 30.4 to 37.0 μg/ml. In contrast, MIC range of 30.6 to 43.0 μg/ml and 55.4 to 71.0 μg/ml were recorded for ethanol and water extract of *B. ferruginea* and *A. conyzoides* respectively.

The purified ethyl galate isolated from dried pod of *Caesalpinia spinosa* intensified β-lactam susceptibility in methicillin resistant and methicillin sensitive strains of *S. aureus*. The maximum activity of alkyl galate against MRSA and MSSA strains occurred at 1-nonyl and 1-decyl galate with an MIC$_{90}$ of 15.6 μg/ml. At concentration lower than the MIC, alkyl galate synergistically elevated the susceptibility of MRSA and MSSA strains to β-lactams (Shibata et al., 2005).

Extracts from various organs of 25 plants of Brazilian traditional medicine were
assayed with respect to their anti-bacterial activities against *E. coli*, a susceptible strain of *S. aureus* and two resistant strains of *S. aureus* harbouring the efflux pumps NorA and MsrA. Amongst the 49 extracts studied, 14 presented anti-bacterial activity against *S. aureus*, including the ethanolic extracts of *Jatropha elliptica* (rhizome), *Schinus terebinthifolius* (stem bark), *Erythrina mulungu* (stem bark), *Caesalpinia pyramidalis* (stem) and *Serjania lethalis* (leaves), and from the stem bark and leaves of *Lafoensia pacari* respectively (de Lima et al., 2006).

Chukwujeckwu et al. (2006) isolated and identified the biologically active component, emodin from the ethanolic root extract of *Cassia occidentalis*. It is inhibitory for *Bacillus subtilis* and *S. aureus*, while inactive against two Gram-negative bacteria (*Klebsiella pneumoniae* and *E. coli*). Similarly, two anthraquinones, zenkequinones A and B, isolated from the stem bark of *Stereospermum zenkeri* were effective against six multiresistant strains of pathogens. Zenkequinone B showed the best antibacterial activity (MIC 9.50 μg/ml) against Gram-negative *Pseudomonas aeruginosa* (Lenta et al., 2007).

*In vitro* efficacy of 15 medicinal plant extracts against multi drug resistant enteric bacteria had been reported at our laboratory. The extracts of *Acorus calamus*, *Hemidesmus indicus*, *Holarrhena antidysenterica* and *Plumbago zeylanica* demonstrated promising activity. Acetone fractions exhibited high potency as compared to ethyl acetate and ethanol fractions. Some of the extracts showed synergistic interaction with antibiotics, tetracycline and ciprofloxacin. TLC bioautography showed the presence of alkaloids, phenols, and flavonoids as active constituents (Aqil and Ahmad, 2007).

Hoque et al. (2007) investigated the antibacterial activity of guava (*Psidium guajava*) and neem (*Azadirachta indica*) extracts against 21 strains of foodborne pathogens viz. *Listeria monocytogenes* (five strains), *S. aureus* (four strains), *E. coli* O157:H7 (six strains), *Salmonella enteritidis* (four strains), *Vibrio parahaemolyticus*, *Bacillus cereus* and five food spoilage bacteria: *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Alcaligenes faecalis* and *Aeromonas hydrophila* (two strains). Guava and neem extracts showed higher antimicrobial activity against Gram-positive bacteria
compared to Gram-negative bacteria except for *V. parahaemolyticus, P. aeruginosa,* and *A. hydrophila.*

Gonçalves et al. (2008) screened the antimicrobial effect of essential oil and methanol, hexane, ethyl acetate extracts from guava leaves. The extracts were tested against diarrhea-causing bacteria: *S. aureus, Salmonella* spp. and *E. coli.* Of the bacteria tested, *S. aureus* strains were inhibited most by the extracts. The methanol extract showed greatest bacterial inhibition. The essential oil showed inhibitory activity against *S. aureus* and *Salmonella* spp.

The ether, methanolic and aqueous extracts of lyophilized rambutan (*Nephelium lappaceum* L.) peels and seeds were evaluated for phenolic contents, antioxidant and antibacterial activities. High amounts of phenolic compounds were found in the peel extracts and the highest content was in the methanolic fraction (542.2 mg/g dry extract). All peel extracts exhibited antibacterial activity against five pathogenic bacteria. The most sensitive strain, *Staphylococcus epidermidis,* was inhibited by the methanolic extract (MIC 2.0 mg/ml) (Thitilertdecha et al., 2008).

Moreover, the antimicrobial activity of traditional Ghanaian medicines was evaluated with special interest against methicillin-resistant *S. aureus* (MRSA). Here, chloroform, ethanol and aqueous extracts of these plants were prepared and agar-well diffusion tests, MIC's and MBC's were used to investigate antimicrobial activity. Extracts of 13 plant species inhibited the growth of one or more of the following bacteria: MRSA, MSSA, *Streptococcus pyogenes, E. coli, P. aeruginosa* and *Proteus vulgaris.* Extracts from 11 of these 13 plant species also inhibited the growth of three or more of 14 additional clinical isolates of MRSA. Aqueous extracts of *Alchornea cordifolia* were active against all 21 bacterial strains tested and showed the highest levels of antibacterial activity with MIC's against MRSA in the range of 1.6-3.1 mg/ml and MBC's in the range of 6.3-12.5 mg/ml (Pesewu et al., 2008).

Khan et al. (2009) tested extracts of five plants against multidrug resistant (MDR) strains of *E. coli, Klebsiella pneumoniae, Streptococcus mutans, S. aureus, Enterococcus faecalis, Streptococcus bovis, P. aeruginosa, S. typhimurium,* and *Candida albicans.* The MDR strains were sensitive to *Acacia nilotica, Syzygium*
aromaticum and Cinnamomum zeylanicum, while the extracts of Terminalia arjuna and Eucalyptus globules were found to be inactive.

Recently, seventeen Thai medicinal plants showed anti-MRSA activity. Among these, Garcinia mangostana was identified as the most potent plant, and its activity was traced to the prenylated xanthone, α-mangostin (MIC and MBC values of 1.95 and 3.91 μg/ml), respectively (Chomnawang et al., 2009).

Similarly, essential oils (EOs) of three Apiaceae species, Bunium persicum, Cuminum cyminum and Carum copticum, extracted by hydrodistillation, were analyzed by gas chromatography (GC) and GC/mass spectrometry. The antibacterial effects of the EOs were assessed on several food-borne pathogens including S. aureus, B. cereus, E. coli O157:H7, S. enteritidis, and Listeria monocytogenes. The ranges of MICs were 0.03–0.5, 0.18–3.0, and 0.37–3.0 mg/ml, for C. copticum, B. persicum and C. cyminum respectively. Moreover, the combination of B. persicum and C. cyminum EOs confirmed synergistic and additive activities against the pathogens (Oroojalian et al., 2010).

2.2.2. Major groups of antibacterial phytocompounds

Plants are solar powered biochemical factories which produce a large array of biological active metabolites that accumulates and are extractable. These plants organic compounds are classified as primary or secondary metabolites (Applezweig, 1980). Secondary metabolites are biologically active compounds frequently present in small quantities compare to primary metabolites. Although large quantities of secondary metabolites are not usually required due to their very strong biological activity and their selection by the external pressure during evolution, they are accumulated in plants due to continuous stimulation (Farnsworth and Bingel, 1977).

Most secondary compounds function in defense against predators and pathogens. Some terpenoids give plants their odour; other (quinones and tannins) are responsible for plant pigments. Major group of phytocompounds and bioactive constituents of several plants have been described and documented by various authors (Chopra et al., 1992; Harborne and Baxter, 1995; Dixon, 2001; Simões et al., 2008; Mahady et al., 2008). Some of the representative groups of antimicrobially active phytocompounds
are described below and also listed in table R1. These can be broadly classified as (i) phenols and polyphenols, (e.g. cinnamic acid, caffeic acid, eugenol, gallic acid, catechol, pyrogallol etc); (ii) Quinones (e.g. plumbagin, lawsone, jaglone, emodin, zenkequinones etc); (iii) Tannins (e.g. corilagin, tellimagrandin I, procyanidine B-2 and pentagalloylgucose etc); (iv) Coumarins (e.g. umbelliferone, angelicin, warferin, cajanuslactone etc); (v) Flavonols, flavones and flavonoids (e.g. epicatechin gallate and epigallocatechin gallate, myricetin, quercetin phloretin, galangin, catechin, flavone); (vi) Terpenoids and essential oils (e.g. camphor, limonene, abscissic acid, aucubin, gossypol, gibberellic acid, β-carotene, menthol, eugenol, carvacrol, thymol etc); (vii) glycosides (e.g. steroidal glycosides, saponin glycosides, anthracene glycoside, cyanogen glycosides, flavonoid glycosides and resinous glycosides, Luteolin, Luteolin 3'-O-β-D-Glucopyranoside, and Luteolin 4’-0-β-D-Glucopyranoside, conessine etc) and (viii) alkaloids (e.g. atropine, codeine, morphine, vincristine, Berberine, piperine, reserpine etc) as reviewed by (Iyenger, 1985; Cowan, 1999; Gurib-Fakim, 2006).
<table>
<thead>
<tr>
<th>Phytochemicals / Group of phytochemicals</th>
<th>Target bacteria</th>
<th>Mechanism of action</th>
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<td>Allicin</td>
<td><em>Salmonella typhimurium</em></td>
<td>Intracellular interaction with thiols, inhibition of RNA synthesis</td>
<td>Feldberg <em>et al.</em>, 1988; Miron <em>et al.</em>, 2000</td>
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<tr>
<td>Berberine</td>
<td><em>S. aureus</em>; <em>K. pneumoniae</em>; <em>S. typhimurium</em>; <em>P. aeruginosa</em></td>
<td>Interaction with cytoplasmic membrane and DNA; Efflux inhibitory activity</td>
<td>Jennings and Ridler, 1983; Stermitz <em>et al.</em>, 2000; Ball <em>et al.</em>, 2006</td>
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<tr>
<td>Glycosides: Luteolin; Saponin glycosides</td>
<td><em>B. cereus</em>; <em>L. plantarum</em>; <em>E. coli</em>; <em>H. pylori</em></td>
<td>Interactions with cell membrane and permeabilization; Inhibition of arylamine N-acetyltransferase</td>
<td>Tsou <em>et al.</em>, 2001; Abbas and Zayed, 2005; Kumarasamy <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>Carvacrol</td>
<td><em>B. cereus</em></td>
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<td>Ultee <em>et al.</em>, 1999</td>
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<tr>
<td>Carvacrol; Thymol</td>
<td><em>E. coli</em>, <em>P. aeruginosa</em>; <em>S. aureus</em>; <em>S. typhimurium</em></td>
<td>Interactions with cell membrane and permeabilization</td>
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<td>Epicatechin gallate; Epigallocatechin gallate</td>
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<td>Gibbons <em>et al.</em>, 2004; Zhang and Rock, 2004</td>
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<tr>
<td>Chlorogenic acid, Rutin and other phenolics</td>
<td><em>E. coli</em>; <em>S. typhimurium</em>; MRSA</td>
<td>Interactions with cell membrane and permeabilization; Efflux inhibition</td>
<td>Fang <em>et al.</em>, 2008; Lou <em>et al.</em>, 2010</td>
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<td>Essential oils</td>
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<td><em>Salmonella spp.</em></td>
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<td></td>
<td><em>Proteus vulgaris</em>, <em>S.</em></td>
<td>DNA synthesis inhibition in</td>
<td>Mori <em>et al.</em>, 1987; Cushnie</td>
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<tr>
<td>Category</td>
<td>Microorganism(s)</td>
<td>Effect</td>
<td>References</td>
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<td>Coumarins</td>
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<td>Piperine</td>
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<td>Quercetin</td>
<td><strong>E. coli</strong></td>
<td>permeability and dissipation of the membrane potential; GyrB subunit of DNA gyrase binding and inhibition of enzyme’s ATPase activity</td>
<td>Mirzoeva <em>et al.</em>, 1997; Plaper <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Abietane diterpenoids</td>
<td><strong>MRSA; VRE</strong></td>
<td>Alterations of membrane permeability; disruption of cytoplasmic membranes</td>
<td></td>
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<tr>
<td>Acylated triterpenoid</td>
<td><strong>B. megaterium; S.</strong></td>
<td>Interference on catabolic enzymes</td>
<td>Babu-Sinha <em>et al.</em>, 1997;</td>
</tr>
<tr>
<td>Saponins</td>
<td>Species</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>Bisglycoside saponins</td>
<td><em>typhimurium</em></td>
<td>and electron transport chain</td>
<td>Mandal <em>et al.</em>, 2005</td>
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<tr>
<td>(Acaciaside A and B)</td>
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<tr>
<td>Sesquiterpenoids: <em>cis</em>-and</td>
<td><em>S. aureus</em>; <em>E. coli</em>; <em>P.</em></td>
<td>Alterations of membrane permeability; disruption of cytoplasmic membranes</td>
<td>Brehm-Stecher and Johnson, 2003; Simões <em>et al.</em>, 2008</td>
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<tr>
<td><em>trans</em>-nerolidol; Farnesol;</td>
<td><em>auruginosa</em></td>
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<td>Bisabolol; Germacrene</td>
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<tr>
<td>Totarol</td>
<td><em>S. aureus</em></td>
<td>Bacterial transport inhibition, disruption of membrane phospholipid, efflux pump inhibition</td>
<td>Haraguchi <em>et al.</em>, 1996; Micol <em>et al.</em>, 2001; Smith <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>Triterpenoid saponins</td>
<td></td>
<td>Membrane permeabilization</td>
<td>Melzig <em>et al.</em>, 2001</td>
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<td>Aliphatic α, β-unsaturated</td>
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<td>aldehydes</td>
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<td>Monoterpenes (1,8-Cineole;</td>
<td><em>S. aureus</em>; <em>E. coli</em></td>
<td>Cell membrane permeabilization</td>
<td>Cox <em>et al.</em>, 2000; Carson <em>et al.</em>, 2002</td>
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<td>Terpinen-4-ol; α-Terpineol)</td>
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<td>3,5-Dihydroxy-4-ethyl-</td>
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<td>Sundar and Chang, 1992</td>
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<td><em>trans</em>-stilbene</td>
<td><em>E. coli</em>, <em>M. luteus</em>, <em>P.</em></td>
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<td><em>vulgaris</em>, <em>P. aeruginosa</em>,</td>
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<td>Tannins: Corilagin and</td>
<td>MRSA</td>
<td>Efflux inhibitory activity</td>
<td>Hatano <em>et al.</em>, 2005</td>
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<td>Tellimagrandin I</td>
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<td>Quinones: Jaglone; Plumbagin</td>
<td><em>E. coli</em>; <em>B. subtilis</em>; <em>S.</em></td>
<td>R plasmids elimination; Interactions with cell membrane and permeabilization</td>
<td>Ali, 1996; Beg and Ahmad, 2000; Chukwujeckwu <em>et al.</em>, 2006; Mahady <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Lawzone</td>
<td><em>aureus</em></td>
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*Partially adapted from (Cowan, 1999 and Simões *et al.*, 2009)
2.3. Free radicals and antioxidants: Significance in disease and health

Antoine Lavoisier (1743-1794), a pioneer oxygen chemist, had pointed out about 150 years ago that animals that respire are the true combustible bodies that burn and consume themselves (Lehninger et al., 1990). The biological combustion produces harmful intermediates called free radicals. A free radical is simply defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. It may be superoxide \( \text{O}_2^* \), oxygen centered radical, thyl \( \text{RS}^* \), sulphur centered radical, trichloromethyl \( \text{CCl}_3^* \), a carbon centered radical or nitric oxide \( \text{NO}^* \) in which the unpaired electron is delocalized between both atoms. The \( \text{O}_2^* \), hydroxyl radicals \( \text{HO}^* \) and other reactive oxygen species (ROS) such as \( \text{H}_2\text{O}_2 \) are continuously produced \textit{in vivo} (Devasagayam et al., 2004).

Active oxygen species also known as reactive oxygen species some of them have unpaired electrons and are free radicals but others are not. These free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants and pesticides, etc (Tiwari, 2001). The production of various active oxygen species are listed below

Table R2 Production of active oxygen species

<table>
<thead>
<tr>
<th>Active oxygen species</th>
<th>Formation</th>
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</thead>
<tbody>
<tr>
<td>Superoxide (Hydroperoxyl radical) ( \text{O}_2^* ) ( \text{HO}_2^* )</td>
<td>Enzymatic and non-enzymatic one electron reduction of oxygen ( \text{O}_2 + e^- \rightarrow \text{O}_2^- \leftrightarrow \text{HO}_2^* (pK = 4.8) )</td>
</tr>
<tr>
<td>Hydroxyl radical, ( \text{HO}^* )</td>
<td>Radiolysis of water, metal-catalyzed decomposition of hydrogen peroxide, interaction of ( \text{NO} ) and superoxide ( \text{H}^+ )</td>
</tr>
<tr>
<td>Alkoxyl and peroxy radicals ( \text{LO}^* ), ( \text{LO}_2^* )</td>
<td>Metal-catalyzed decomposition of hydroperoxides</td>
</tr>
<tr>
<td>*Hydrogen peroxide, ( \text{H}_2\text{O}_2 )</td>
<td>Dismutation of superoxide, oxidation to</td>
</tr>
</tbody>
</table>
Review of Literature

*Iron-oxygen complex, Fe=O, etc.

*Singlet oxygen complex, \(^1\text{O}_2\)

Lipid and protein hydroperoxides

Nitrogen dioxide, \(\text{NO}_2^-\)

Nitric oxide, \(\cdot \text{NO}\)

Thiyl radical, \(\text{RS}^-\)

Protein radical

sugars

Hemoglobin, myoglobin, etc.

Photosensitized oxidation, bimolecular interaction between peroxyl radicals, reaction of hypochlorite and hydrogen peroxide

Oxidation of lipids and proteins

Reaction of peroxyl radical and NO, polluted air and smoking

Nitric oxide synthase, nitroso thiol, and polluted air

Hydrogen atom transfer from thiols

Hydrogen atom transfer from protein

* Non radicals (Noguchi and Niki, 1999)

These exogenous pollutants generating free radicals have become part and parcel of our daily inhaling/ingesting life and infact there appears no escape from them. Continuous interaction of the animal physiological systems with these free radicals generated either indigenously or inhaled/ingested from exogenous sources. Therefore, excess load of free radicals cause cumulative damage of protein, lipid, DNA, carbohydrates and membrane, resulting in so-called oxidative stress. Therefore, living creature has evolved a highly complicated defense system with antioxidants composed of enzymes and vitamins against oxidative stress in the course of their evolution (Valko et al., 2007).

It is a free radical entity, which is primarily responsible to initiate damage to the biological targets leading to different disorders. The antioxidants may be classified on the basis of their mode of action as free-radical scavenger terminator, chelator of metal ions, capable of catalyzing lipid peroxidation or as oxygen scavengers that react with superoxides and so on (Sree Kumar et al., 2002).

Research in the recent past has accumulated enormous evidences revealing that enrichment of body systems with natural antioxidants may correct the vitiated
homeostasis (Halliwell, 1994; Bruckdorfer, 1996; Tiwari, 1999; Pietta, 2000) and can prevent the onset as well as treat diseases caused and or fostered due to free radical mediated oxidative stress. As one of the aspects of the body’s natural ecosystem, it is increasingly being realized now that a majority of the diseases/disorders are mainly due to the imbalance between prooxidant and antioxidant homeostatic phenomenon in the body. Prooxidant conditions dominate either due to increased generation of free radicals and or their poor quenching/scavenging into the body (Dringen, 2000).

Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects (Nose, 2000; Finkel and Holbrook, 2000). Thus the antioxidants status in human reflects the dynamic balance between the antioxidant defense and prooxidant conditions and has been suggested as a useful tool is estimating the risk of oxidative damage (Nose, 2000; Polidori, 2001). Possible ways of balance of ROS generation and antioxidant defense may be summarized below.

Balance of ROS generation and antioxidant defense
2.3.1. Antioxidants and their mode of action

In food, antioxidants have been defined as a substance that in small quantities is able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats (Chipault, 1962; Halliwell, 1990). However, in biological systems the definition for antioxidants has been extended to any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate like lipids, proteins, DNA, and carbohydrates.

Antioxidants may exert their effects by different mechanisms, such as suppressing the formation of active species by reducing hydroperoxides (ROO•) and H₂O₂ and also by sequestering metal ions, scavenging active radicals, repairing and/or clearing damage. Similarly some antioxidants also induce the biosynthesis of other antioxidants or defense enzymes. The bioactivity of an antioxidant is dependent on several factors like their structural criteria, physico-chemical characteristics and in vivo radical generating conditions (Tiwari, 2001).

An antioxidant works by retarding the oxidation. In biology, oxidation is often started by free radicals. The role of an antioxidant is to intercept a free radical before it can react with the substrate e.g. phenol (AOH), the reaction of interest with ROO• is:

\[ \text{AOH} + \text{ROO•} \rightarrow \text{AO•} + \text{ROOH} \]

Based on several theoretical models and complex calculations; Wright (2003) concluded that bond dissociation enthalpy (BDE) gives excellent correlation for this requirement with many known families of antioxidants, like vitamins E and C, resveratrol, galloカテchins, ubiquinol, etc. Major understanding of beneficial therapeutic activities of antioxidants has arisen with studies on vitamins E and C and ubiquinol Q_{10} that serve as excellent reference material.

Concurrently, Gao et al. (1999) suggested that the free radical scavenging properties of antioxidant compounds are often associated with their ability to form stable radicals after their reaction with free radicals. Apart from the above criteria, there are other features, which could also play an important role in considering antioxidant properties such as (i) Rate constant with different types of radicals as argued by De Groot and Rauen (1998). (ii) Stoichiometry of the radical scavenging process. (iii)
Effective concentration to be reached at the site where the reactive species is being formed. (iv) Stability and decay kinetics of the resulting product such as flavonoid aroxy radical; is also an important aspect.

The mechanism of antioxidative action is indicated below:

**Initiation:**
\[ \text{LH} + \text{O}_2 \rightarrow \text{L}^- + \cdot\text{O}_2 \text{H} \]
\[ \text{LH} \rightarrow \text{L}^- + \text{H}^- \]

**Propagation:**
\[ \text{L}^- + \text{O}_2 \rightarrow \cdot\text{O}_2 \text{L}^- \]
\[ \cdot\text{O}_2 \text{L}^- \rightarrow \text{LOOH} + \text{L}^- \]

**Termination:**
\[ \text{L}^- + \text{L}^- \rightarrow \text{LL} \]
\[ \text{L}^- + \cdot\text{O}_2 \text{L}^- \rightarrow \text{LOOL} \]
\[ \cdot\text{O}_2 \text{LOO}^- + \cdot\text{O}_2 \text{LOO}^- \rightarrow \text{LOOL} + \text{O}_2 \]

For convenience, antioxidants have been traditionally divided into two classes; primary or chain-breaking antioxidants, and secondary or preventive antioxidants (Madhavi *et al.*, 1996).

Chain-breaking mechanisms are represented as follows:

\[ \text{L}^- + \text{AH} \rightarrow \text{LH} + \text{A}^- \] (1)
\[ \cdot\text{O}_2 \text{L}^- + \text{AH} \rightarrow \text{LOOH} + \text{A}^- \] (2)
\[ \cdot\text{O}_2 \text{LOO}^- + \text{AH} \rightarrow \cdot\text{O}_2 \text{LOOH} + \text{A}^- \] (3)

Thus, radical initiation (by reacting with a lipid radical: \(\text{L}^-\)) or propagation (by reacting with alkoxy: \(\cdot\text{O}_2 \text{L}^-\) or peroxy: \(\cdot\text{O}_2 \text{LOO}^-\) radicals) steps are inhibited by the antioxidant: \(\text{AH}\).

On the other hand, secondary (preventive) antioxidants retard the rate of oxidation. For example, metal chelators (e.g., iron-sequesterants) may inhibit Fenton-type reactions that produce hydroxyl radicals (Ames *et al.*, 1993).

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} \]
2.3.2. *In vitro* methods for determination of antioxidant activity of natural products

There are several *in vitro* and *in vivo* methods available to determine the antioxidant activity of plant extracts and natural compounds. These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods particular antioxidants have varying contributions to total antioxidant potential (Prior *et al.*, 2005). The details of such methods are described elsewhere (Prior *et al.*, 2005; Ali *et al.*, 2008). Some of the commonly used methods include DPPH (2,2-diphenyl-1-picrylhydrazyl), ORAC (oxygen radical absorbance capacity), TEAC (trolox equivalent antioxidant capacity), FRAP (ferric reducing antioxidant power), Phosphomolybdenum, TRAP (total reactive antioxidant potential), ABTS\(^{+}\) (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), CUPRAC (cupric ion reducing antioxidant capacity) etc. The four methods used in this study are briefly described below.

2.3.2.1. DPPH assay for determination of antioxidant activity

DPPH assay is commonly used in primary screening of phytocompounds. Diphenyl picryl hydrazyl (DPPH) is nitrogen centred stable radical. It reacts similar to the peroxyl radical. Its reaction rates correlate directly with antioxidant activity. Higher the rate, more effective is the antioxidant (Wright, 2003). Two mechanisms for antioxidants to scavenge DPPH radical have been proposed (Wang and Zhang, 2003)

The first is a direct H-atom abstraction process [eq. (1)] and the second is a proton concerted electron transfer process [eq (2)].

\begin{align*}
1 & \text{DPPH}^{+} + \text{RXH} \rightarrow \text{DPPHH} + \text{RX}^{+}, \\
2 & \text{DPPH} + \text{RXH} \rightarrow \text{DPPH}^{+} + \text{RXH}^{+} \rightarrow \text{DPPHH} + \text{RX}^{-}.
\end{align*}

where X represents O, N, S or C. First pathway is governed to a larger extent by X–H bond dissociation energy (BDE), of RXH and DPPHH. Only if the BDE of former is lower than that of the latter, the reaction is permitted. The BDE for DPPHH is calculated to be 172.22 Kcal/mol.
While the second pathway is determined by ionization potentials (IP) of RXH and DPPH. The prerequisite for this reaction to proceed is that, IP of the RXH should be lower than that of DPPH\(^-\). The IP for DPPH\(^-\) is observed to be 59.60 Kcal/mol. Phenol, amino, or thiophenol groups are commonly known to be the active groups for scavenging DPPH.

Antioxidants tested on DPPH were also found extremely effective in cell systems of oxidative stress used to test anticancer agents (Wright, 2003). This simple test further provides information on the ability of a compound to donate electrons, the number of electrons a given molecule can donate and on the mechanism of antioxidant action. Furthermore, in case where structure of the electron donor is not known (e.g., plant extract), this method can afford data on the reduction potential of the sample, and hence can be helpful in comparing the reduction potential of unknown materials. Vaya (2003) observed that compounds which were able to donate electrons to the DPPH molecule were the same as those that showed high activity in inhibiting LDL oxidation induced under different conditions. The test is simple and rapid and needs only a UV-Vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening. However, interpretation is complicated when the test compounds have spectra that overlap DPPH at 515 nm (Noruma et al., 1997).

2.3.2.2. CUPRAC (Cupric ion reducing antioxidant capacity) method

The chromogenic redox reagent used for the CUPRAC assay was bis (neocuproine) copper (II) chelate. This reagent was useful at pH 7, and the absorbance of the Cu(I)-chelate formed as a result of redox reaction with reducing polyphenols was measured at 450 nm (Apak et al., 2004). The chromogenic oxidizing reagent of the developed CUPRAC method, i.e., bis (neocuproine) copper (II) chloride (Cu(II)-Nc), reacts with n-electron reductant antioxidants (AO) in the following manner:

\[
\begin{align*}
n \text{Cu(Nc)}_2^{2+} + n\text{-electron reductant (AO)} & \leftrightarrow n \text{Cu(Nc)}_2^{+} + n\text{-electron oxidized product} \\
& + n \text{H}^+ \\
\end{align*}
\]

In this reaction, the reactive Ar-OH groups of polyphenolic antioxidants are oxidized to the corresponding quinones (Ar=O) and Cu(II)-Nc is reduced to the highly colored Cu(Nc)_2^+ chelate showing maximum absorption at 450 nm. Although the
concentration of Cu$^{2+}$ ions was in stoichiometric excess of that of neocuproine in the CUPRAC reagent for driving the redox equilibrium reaction represented by (Eq. 1) to the right, the actual oxidant was the Cu(Nc)$_2$$^{2+}$ species and not the sole Cu$^{2+}$, because the standard redox potential of the Cu(II/I)-neocuproine was 0.6 V, much higher than that of the Cu$^{2+}$/Cu$^{+}$ couple (0.17 V) (Apak et al., 2008). As a result, polyphenols were oxidized much more rapidly and efficiently with Cu(II)-Nc than with Cu$^{2+}$, and the amount of colored product (i.e., Cu(I)-Nc chelate) emerging at the end of the redox reaction was equivalent to that of reacted Cu(II)-Nc. The liberated protons are buffered in ammonium acetate medium. The CUPRAC method was shown to be free from the chemical deviations from Beer's law, as demonstrated on synthetic and real solutions. The total antioxidant capacity of a synthetic antioxidant mixture is the sum of the capacities of individual constituents of the mixture. There was also a good parallelism of linear curves for each antioxidant in pure aqueous solution and in real complex mixtures like fruit juices and herbal teas.

2.3.2.3. FRAP (Ferric reducing antioxidant power) assay

The FRAP assay was originally developed by Benzie and Strain (1996) to measure reducing power in plasma. The reaction measures reduction of ferric-2,4,6-tripyridyl-s-triazine (TPTZ) to a colored product. The reaction detects compounds with redox potentials of <0.7 V (the redox potential of Fe$^{3+}$-TPTZ), so FRAP is a reasonable screen for the ability to maintain redox status in cells or tissues. Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols (Pulido et al., 2000). The redox potential of Fe (III)-TPTZ is comparable with that of ABTS$^+$ (0.68V). Similar compounds react in, both the TEAC and FRAP assays. The FRAP mechanisms is totally electron transfer rather than mixed SET and HAT, so in combination with other methods it can be very useful in distinguishing dominant mechanisms with different antioxidants.

$$\text{Fe}^{3+}\cdot\text{TPTZ} + \text{reducing antioxidant} \rightarrow \text{Fe}^{2+}\cdot\text{TPTZ} \text{ (intense blue at 595 nm)}$$

In contrast to other tests of total antioxidant power, the FRAP assay is simple, speedy, inexpensive, and robust and does not require specialized equipment. The FRAP assay
can be performed using automated, semi-automated, or manual methods (Benzie and Strain, 1999).

2.3.2.4. Phosphomolybdenum method

It is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Kanner et al., 1994). It is a simplified procedure described for the determination of inorganic phosphate in body fluids. This method employs two stable reagents and requires minimum number of steps. The phosphomolybdate formed is immediately reduced in situ by the ferrous ion to produce a blue color that is stable for several hours. The intensity of color is insensitive to changes in concentration of acid, molybdate, ferrous ion, and thiourea, and to losses in decanting the serum supernatant (Goldenberg and Fernandez, 1966).

2.3.3. Antioxidants activity of plant extracts and phytocompounds

It has now been established that some common pathways in pathogenesis of different diseases mediated through oxidative stress, free radicals and various kind of radicals like hydroxyl, alkoxy, peroxyl and carbon centred have been observed to be involved in oxidative stress in vivo. Niki et al. (1995) suggested that the peroxyl radicals should be the major target radical for radical scavenging antioxidants in vivo. Phytocompounds like flavonoids, flavanols, present in abundance in dietary constituents, contain a strong nucleophilic center that reacts with electrophillic species and thereby decreases the bioavailability of the ultimate carcinogens (Yang and Wang, 1993). Therefore, nucleophillic electron donating species present in tea may be one of the important mechanisms of action involved in inhibition of carcinogenesis, where electrophillic carcinogenic species may be trapped by nucleophillic polyphenols (Smith et al., 1995).

Considering these criteria and antioxidant property as the base in view, several novel molecules have emerged for development as therapeutics for various diseases like atherosclerosis (Cynshi, 1998; Loo, 2003) and simultaneously, several insights have
been provided for therapeutic advancement for Alzheimer’s and Parkinson’s disease (Behl, 1999; Esposito, 2002).

Currently, there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Traditional herbal medicines form an important part of the healthcare system of India (Scartezzini and Speroni, 2000; Nedyalka et al., 2006; Ali et al., 2008; Krishnaiah et al., 2010). Therefore, by applying various antioxidant methods, several authors have screened potential plants with rich content of antioxidants and phytocompounds from different part of the world (Kikuzaki et al., 1991; Jitoe et al., 1992; Rice-Evans et al., 1996; Vani et al., 1997; Surh, 1999; Lee and Shikamoto, 2000). Some of the relevant literature published in the last 10 years is briefly reviewed here

Ruberto and Baratta (2000) studied green tea catechins and found out that (−) epicatechin (EC), (−) epigallocatechin (EGC), (−) epicatechin gallate (ECG), (−) epigallocatechin gallate (EGCg) and trolox inhibited the decreases of apolipoprotein B-100 (apo B) and α-tocopherol in a radical reaction of human plasma initiated by Cu$$^{2+}$$ where the concentrations of EC, EGC, ECG, EGCg, and Trolox for 50% inhibition ($$IC_{50}$$) of apo B fragmentation were found to be 39.1, 42.2, 14.6, 21.3 and 36.2 μM respectively. These results demonstrated that tea catechins serve as an effective antioxidant in plasma and that the gallate group has a strong antioxidant activity.

On the other hand, Shobana and Naidu (2000) investigated the potential antioxidant activities of selected spices extracts like garlic, ginger, onion, mint, cloves, cinnamon and pepper in equal ratio of water and ethanol. Among these spices, cloves exhibited highest while onion showed least antioxidant activity. The relative antioxidant activity decreased in the order of cloves, cinnamon, pepper, ginger, garlic, mint and onion respectively.
Russo et al. (2001) investigated the free radical scavenging capacity of methanolic extracts from Celastrus paniculatus L. (Celastraceae), Picrorhiza kurroa L. (Scrophulariaceae), Withania somnifera L. (Solanaceae) and the effect on DNA cleavage induced by H$_2$O$_2$, UV photolysis and reported that these extracts showed a dose dependent free radical scavenging capacity and a protective effect on DNA cleavage. In the same year, Lee and Shibamoto obtained the aroma extract from dried clove buds. Twenty two compounds were identified in the extracts of clove buds by gas chromatography and gas chromatography/mass spectrometry. The antioxidant activity of clove bud extract and its major components, eugenol and eugenyl acetate, was comparable to that of the natural antioxidant, α-tocopherol (vitamin E).

Noda et al. (2002) demonstrated the antioxidant activities of freeze dried preparations of a 70% acetone extract of pomegranate (Punica granatum) and its three major anthocyanidins (delphinidin, cyanidin and pelargonidin). Free radical scavenging activities were examined using an ESR technique. The ID$_{50}$ values of delphinidin, cyanidin and pelargonidin were 2.4, 22 and 456 μM, respectively.

Water, aqueous-methanol, and aqueous-ethanol extracts of freeze dried leaves of Moringa oleifera from different agroclimatic regions demonstrated good radical scavenging capacities using DPPH method. The major bioactive compounds of phenolics were found to be flavonoid groups such as quercetin and kaempferol (Siddhuraju and Becker, 2003).

The volatile oil from cinnamon fruit stalks was analyzed with GC and GC-MS and screened for its potential as an antioxidant using β-carotene-linoleate and phosphomolybdenum complex method in vitro. The volatile oil showed 55.94% and 66.9% antioxidant activity at 100 and 200 ppm concentrations, respectively. They also showed good antioxidant capacity when tested by formation of the phosphomolybdenum complex (Jayaprakasha et al., 2003).

Thinunavukkarasu et al. (2003) investigated the effect of aqueous extract of fenugreek seeds (Trigonella foenumgraecum) on lipid peroxidation and antioxidant status in experimental ethanol toxicity in rats. The seeds exhibited appreciable antioxidant property in vitro which was comparable with that of reduced glutathione and α-
tocopherol. Similarly Oktay et al. (2003) reported that the water and ethanol extracts of fennel (*Foeniculum vulgare*) seeds showed strong antioxidant activity by various antioxidant methods. A 100 μg of water and ethanol extracts exhibited 99.1% and 77.5% inhibition of peroxidation in linoleic acid system, respectively, and greater than the same dose of α-tocopherol (36.1%). The both extracts of fennel seeds had effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities.

Wangensteen et al. (2004) investigated the antioxidant activity of different extracts from leaves and seeds of coriander (*Coriandrum sativum*) and coriander oil. Positive correlations were found between total phenolic content in the extracts and antioxidant activity. Ethyl acetate extract contributed to the strongest activity and it was even more in leaves when compared with seeds. In another study, Melo et al. (2004) identified the four fractions from the aqueous extracts of coriander using chromatography in a silica gel column. Their antioxidant activity, according to the β-carotene/linoleic acid model, was similar but inferior to that of the crude extract and BHT. Of the phenols identified through gas chromatography and mass spectrometry, it was noted that caffeic acid was present in high concentration (4.34 μg/ml in fraction I and 2.64 μg/ml in fraction III), whereas protocatechinic acid and glycitin were present in high concentration in fraction II (6.43 μg/ml) and fraction IV (3.27 μg/ml), respectively. This study further suggested that phenolic acids are principal components responsible for the antioxidant activity.

Similarly, Qian and Nihorimbere (2004) evaluated the total phenolic content and antioxidant power of different extracts from *Psidium guajava* leaf. Remarkably high total phenolic content in guava leaf 575.3 ±15.5 (ethanol extract) and 511.6 ± 6.2 (water extract) mg of GAE/g of dried weight was reported. They demonstrated that antioxidant potential of commercial and ethanol extracts of guava leaves were at par of ascorbic acid whereas aqueous extracts showed lower antioxidant activity. This study revealed that guava leaf extracts comprised of effective potential source of natural antioxidants.

Dixit et al. (2005) evaluated the antioxidant properties of different fractions of germinated fenugreek seeds. Aqueous fraction of fenugreek exhibited the highest
antioxidant activity which is partly due to the presence of flavonoids and polyphenols. Wong et al. (2006) examined the antioxidant properties of 25 edible tropical plants using DPPH free radical scavenging and reducing ferric ion antioxidant potential (FRAP) assays. They suggested that polyphenols in the extracts were partly responsible for the antioxidant activities. In the same year, Katalinic et al. (2006) analyzed 70* medicinal plant infusions for the total phenolic content by Folin-Ciocaltceu assay and related total antioxidant capacity by FRAP assay. The results indicated that Melissae folium infusions could be an important dietary source of phenolic compounds with high antioxidant capacity comparable with red wine or beverages like tea.

In addition, the structure-radical scavenging activity relationships of a large number of representative phenolic compounds (e.g., flavanols, flavonols, chalcones, flavones, flavanones, isoflavones, tannins, stilbenes, curcuminoids, phenolic acids, coumarins, lignans, and quinones) identified in the traditional Chinese medicinal plants were evaluated using the improved ABTS** and DPPH methods. Different categories of tested phenolics showed significant differences in radical scavenging activity. Tannins demonstrated the strongest activity, while most quitiones, isoflavones, and lignans tested showed the weakest activity. This study suggest that the number and position of hydroxyl groups and the related glycosylation and other substitutions largely determined radical scavenging activity of the tested phenolic compounds (Cai et al., 2006).

Wojdylo et al. (2007) investigated trolox equivalent antioxidant capacities (TEAC) and phenolic contents of 32 spices extracts from 21 botanical families grown in Poland. The total antioxidant capacity was estimated by the following methods: ABTS.⁺ (2', 2'azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)), DPPH. (1,1-diphenyl-2-picrylhydrazyl radical) and ferric reducing antioxidant power (FRAP) expressed as TEAC. A positive relationship between TEAC (ABTS.⁺ and FRAP) values and total phenolic content was observed.

Similarly, the ethanol extracts from 24 plant species commonly found in Thailand were investigated and compared for their antioxidant activity by ABTS assay. The ethanol extract from the leaves of guava (Psidium guajava) showed the highest
antioxidant capacity with the TEAC value of 4.908 ± 0.050 mM/mg, followed by the fruit peels of rambutan (Nephelium lappaceum) and mangosteen (Garcinia mangostana) with the TEAC values of 3.074 ± 0.003 and 3.001 ± 0.016 mM/mg respectively. The further investigation of guava leaf extracts from different solvents indicated the highest antioxidant activity in methanol fraction followed by the butanol and ethyl acetate fractions respectively. The results demonstrated that the mechanism of antioxidant action of guava leaf extracts was free radical scavenging and reduction of oxidized intermediates (Tachakittirungrod et al., 2007).

Antioxidant potential of phenolic compounds from green pepper (Piper nigrum L.) and lignans from fresh mace (Myristica fragrans) were evaluated for their ability to scavenge DPPH radicals, inhibit lipid peroxidation and protect plasmid DNA damage upon exposure to gamma radiation. EC\textsubscript{50} values of the major phenolic compounds of green pepper namely, 3,4-dihydroxyphenyl ethanol glucoside, 3,4-dihydroxy-6-(N-ethylamino) benzamide and phenolic acid glycosides were found to be 0.076, 0.27 and 0.12 mg/ml, respectively, suggesting a high radical scavenging activity of these phenolics (Chatterjee et al., 2007).

Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf were evaluated by cation (ABTS\textsuperscript{•+}, DPPH\textsuperscript{•−}, peroxyl (ORAC) and hydroxyl (HO\textsuperscript{•−}) radicals. The results indicated that these botanical samples might serve as potential dietary sources of natural antioxidants for improving human nutrition and health (Su et al., 2007).

Barks extracts of four different trees (Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam.) in three different solvents 80% methanol, 80% ethanol, and 80% acetone (solvent:water, 80:20 v/v) were evaluated for their antioxidant activity, total phenolic (TP), and total flavonoids (TF) contents. Significant ($P < 0.05$) differences were observed in the TP, TF, inhibition of linoleic acid oxidation and DPPH scavenging activity of different bark extracts. Nevertheless, minute variation was reported in reducing power. The correlation among the results of different antioxidant assays revealed a strong relationship between some of the assays, though, a number of different methods may be necessary to adequately assess the \textit{in vitro} antioxidant activity of a specific plant material (Sultana et al., 2007).
Agbor et al. (2007) demonstrated the polyphenolic concentration and free radical scavenging activity of three *Piper* species (*Piper guineense*, *Piper nigrum* and *Piper umbellatum*). *Piper* extracts exhibited a 57-76% scavenging effect on hydroxyl radical at 5 mg/ml, a 0.4-0.6 reducing power and an 88.3-93.9% metal chelating activity at a dose level of 8 mg/ml of extract.

Similarly, in the same year Saxena and workers attempted to generate a database on the antioxidant activity (AOA) and phenolic content (PC) of different food groups such as cereals, legumes, oil seeds, oils, green leafy vegetables, other vegetables, spices, roots and tubers. A significant correlation was observed between the AOA and PC of the plant foods. The results suggested that phenolics may contribute significantly to the AOA of some plant foods, such as spices and dehusked legumes.

Four (red, violet, white and green) varieties of *Allium cepa* were studied for their total phenolic contents (TPC), antioxidant (AOA) and free radical scavenging activities. The TPC varied from 4.6 to 74.1 mg/g GAE, AOA varied from 13.6% to 84.1% and FRSA showed wide range in terms of IC50 (inhibitory concentration) from 0.1 to 15.2 mg/ml, EC50 (efficient concentration) from 4.3 to 660.8 mg/mg and ARP (antiradical power) from 0.15 to 23.2. The unutilized outer layers of the red variety were a rich source of quercetin (5.1 mg/g) with high AOA, and also showed significant protection of DNA damage caused by free radicals (Prakash et al., 2007).

Reddy et al. (2008) evaluated the antioxidant and antimicrobial properties of hexane, methanol and aqueous extracts of *Soymida febrifuga* (Maliaceae) leaves. The results showed that the methanol and aqueous extracts of leaf had a higher antioxidant activity and total phenolic content. Similarly, high antioxidant activity was found in monoterpenoid extracts (from *Myristica fragrans*) including terpinene-4-ol, alpha-terpineol, and 4-allyl-2, 6-dimethoxyphenol (Maeda et al., 2008).

To search for edible herbal extracts with potent antioxidant activity, a large scale screening on herbs was conducted based on the superoxide scavenging activity. Out of these, the extracts of *Punica granatum* (Peel), *Syzygium aromaticum* (Bud), *Mangifera indica* (Kernel), and *Phyllanthus emblica* (Fruit), directly scavenged the superoxide anions and that was comparable to L-ascorbic acid. Furthermore,
polyphenol determination indicated that the activity was at least in part attributable to polyphenols (Saito et al., 2008).

Gupta and Prakash (2009) identified the concentration dependent antioxidant potential of methanolic extracts of green leafy vegetables (GLV), *Amaranthus* sp., *Centella asiatica*, *Murraya koenigii* and *Trigonella foenumgraecum* in different systems. The maximum DPPH scavenging activity and reducing power was exhibited by *Murraya koenigii*. Multiple regression analysis also showed highly significant relationship of total antioxidant activity, free radical scavenging activity, and reducing power with polyphenol and β-carotene respectively.

Curcumin (diferuoyl methane) is a mixture of three curcuminoids and major component of *Curcuma longa* L. Curcumin inhibited 97.3% lipid peroxidation of linoleic acid emulsion at 15 μg/ml concentrations (20 mM). In addition, curcumin had an effective DPPH scavenging, ABTS⁺ scavenging, DMPD⁺ scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, ferric ions (Fe³⁺) reducing power and ferrous ions (Fe²⁺) chelating activities (Akc and Gulčin, 2008).

Stangeland et al. (2009) analyzed the antioxidant activity (AOA) in fruits and vegetables from Uganda. The dietary plants with highest AOA per serving size were pomegranate (*Punica granatum*), *Canarium schweinfurthii*, guava (*Psidium guajava*), mango (*Mangifera indica*) and tree tomato (*Cyphomandra betacea*) with values ranging from 3.00 to 8.91 mmol/serving.

In the similar way, Prasad et al. (2009) screened five species of *Cinnamomum*, namely *C. burmanni*, *C. cassia*, *C. pauciflorum*, *C. tamala* and *C. zeylanica*, for their antioxidant potentials using various in vitro assays such as total antioxidant capability, DPPH radical scavenging activity, reducing power and superoxide anion scavenging activity at various concentrations. *C. zeylanica* showed the highest DPPH radical scavenging activity, total antioxidant activity and reducing power, while *C. tamala* exhibited the highest superoxide anion scavenging activity. By the analysis of the high performance liquid chromatography coupled to diode array detector (HPLC-DAD), three flavonoids namely quercetin, kaempferol and quercetrin were identified and quantified.
The systematic evaluation of the antioxidant potential of different parts of *Foeniculum vulgare* from Portugal was performed. The shoots had the highest radical-scavenging activity and lipid peroxidation inhibition capacity (EC$_{50}$ values < 1.4 mg/ml), which was in agreement with the highest content in phenolics (65.85 ± 0.74 mg/g) and ascorbic acid (570.89 ± 0.01 µg/g) found in this part. The shoots also revealed high concentration of tocopherols (34.54 ± 1.28 µg/g) which were the only part with flavonoids (Barros *et al.*, 2009).

In the same year, Visavadiya and colleagues evaluated the antioxidant action of aqueous and ethanolic seed extracts from *Sesamum indicum* using various *in vitro* ROS/RNS generated chemical and biological models. The ethanolic extract of *S. indicum* possessed strong antioxidant capacity and offered effective protection against LDL oxidation susceptibility.

The total phenolic content and antioxidant activity of methanolic, ethanolic and aqueous extracts of myrtle (*Myrtus communis*) leaves and berries were measured to find new potential sources of natural antioxidants. The leaf extracts showed higher antioxidant activities than berry extracts, while the overall antioxidant strength was in the order methanol > water > ethanol in leaf extracts and methanol > ethanol > water in berry (fruit) extracts. The phenolic content exhibited a positive correlation with the antioxidant activity: DPPH assay showed the highest correlation ($r^2 = 0.949$), followed by the reducing power assay ($r^2 = 0.914$) and the lowest for the β-carotene linoleic acid assay ($r^2 = 0.722$) (Amensour *et al.*, 2009).

The antioxidant capacities of a wide range of onion cultivars; nine commercial cultivars and five advance selections differing in color were determined. The results suggested that the red onions had higher antioxidant activities than yellow and white onions although yellow onions had the richest phenolic contents (Gökçe *et al.*, 2010).

Ismail *et al.* (2010) investigated the antioxidant activities of the thymoquinone-rich fraction (TQRF) extracted from *Nigella sativa* and its bioactive compound, thymoquinone (TQ), in rats with induced hypercholesterolemia. TQRF and TQ effectively improved the plasma and liver antioxidant capacity and enhanced the expression of liver antioxidant genes of hypercholesterolemic rats.
Cevallos-Casals and Cisneros-Zevallos (2010) studied the impact of germination on phenolic content and antioxidant activity of 13 edible seed species. This study showed that germinated edible seeds are an excellent source of dietary phenolic antioxidants. Similarly Belguith-Hadriche et al. (2010) demonstrated relationship between the lipid effects, the phenolic content, and the antioxidative effects of an ethyl acetate extract of fenugreek. These results revealed significant hypocholesterolemic effects and antioxidant activity in an ethyl acetate extract of fenugreek seed may be partly due to the presence of flavonoids, especially naringenin.

2.4. Mutagenicity and antimutagenicity of medicinal plants

Mutations are the cause of innate metabolic defects in cellular system, triggering the morbidity and mortality in living organisms. A plethora of synthetic and natural substances, apart from various genotoxic physical and biological agents, are known to act as mutagenic, co-carcinogenic and carcinogenic agents. Since, the mutagens are involved in the initiation and promotion of several human diseases including cancer, the significance of novel bioactive phytocompounds in counteracting the promutagenic and carcinogenic effects is now gaining credence. Indeed, the chemicals that reduce the mutagenicity of physical and chemical mutagens are referred as antimutagens (Mitscher et al., 1986).

It has been suggested that use of antimutagens and anticarcinogens in everyday life will be the most effective procedure for preventing human cancers and genetic disorders (Ferguson, 1994). The antimutagens have been first reported almost four decades ago, and since then numerous studies have been carried out in order to identify compounds, which might protect against DNA-damage and its consequences. However, there is increasing awareness that certain naturally occurring substances in plants and other natural sources provide potential protection against environmental mutagens/ carcinogens as well as endogenous mutagens (Sangwan et al., 1998; Shankel et al., 2000). There are continued efforts all over the world to explore the rich biodiversity of edible as well as medicinal herbs and other non-toxic plants in search of most effective phytoantimutagens.
Some of the mechanisms of antimutagenesis and anticarcinogenesis are known (Ferguson, 1994; Ishaq et al., 2003) which includes:

(i) **Bioantimutagens**: These are equivalent to the “true” antimutagens. They act on the repair and replication processes of the mutagen damaged DNA, resulting in a decline in a mutation frequency.

(ii) **Desmutagens**: These must be considered only as apparent antimutagens. They indirectly inactivate the mutagens either fully or partially.

(iii) **Chemical or enzymatic activators**: Many mutagens, which are chemically reactive, acting not only on DNA but also on proteins and enzymes, may be directly inactivated by a range of different chemicals. Antimutagenic and anticarcinogenic properties have been associated with both inhibitors and inducers of cytochrome P-450 enzymes such as indole-3-carbinol. Inducers of phase II enzymes such as glutathione transferase tend to inhibit a wide range of target carcinogens eg isothiocyanates such as benzyl isothiocyanates and antioxidants such as 2,3-tert-butyl-4-hydroxyanisole (BHA).

(iv) **Prevention of formation of active species**: Many genotoxic carcinogens require metabolic activation to an electrophilic form that can react with DNA. Although these processes commonly occur in the liver, there is increasing evidence for metabolic activation by other tissues also, especially of the gastrointestinal tract. N-nitroso compounds are often formed in the stomach through a reaction from nitrite and secondary or tertiary amines.

(v) **Scavenging**: A number of desmutagens are able to scavenge dietary mutagens (activated or non-activated) through binding or adsorption. In general, the mutagen remains intact during this process, but is unable to react with DNA. Chlorophyllin and some dietary fibers appear to act in this way.

(vi) **Antioxidant and free radical scavengers**: A wide range of chemopreventive agents has anti-oxidant or free radical scavenging activity, eg, carotenoids, retinoids and flavonoids. Once formed, free radicals, having a short half-life eg, OH radical (half life $=10^6$ sec), can be removed or inactivated.
Large scale screening with plant extracts have led to the identification of numerous protective phytocompounds (Kada et al., 1982; Kada et al., 1986). Systematic carcinogenicity studies of the plants also led to the possibilities of developing the nutritional strategies that may protect humans against DNA damage and cancer (Wattenberg, 1987). This assumption is also supported by epidemiological studies, which suggested that around 20–60% of all cancers are diet related (Doll, 1992) and that the intake of vegetables and fruits is inversely related to the incidence of various forms of cancer (Steinmetz and Potter, 1996). Computer aided literature search revealed that in the last 25 years, more than 25,000 articles are published on anti-mutagens and anti-carcinogens, and about 80 percent are on plant constituents used as foods or for medical purposes. These “bioactive” compounds belong to a variety of different chemical groups such as phenolics, pigments, allylsulfides, tannins, anthocyanins, flavonoids, phytosterols, protease inhibitors and phytoestrogens. Many of these substances elicit, apart from their antimutagenic and anti-carcinogenic properties, additional beneficial effects such as activation of the immune system and/or protection against cardiovascular diseases (Middelton and Kandaswami, 1993).

2.4.1. Assays for mutagenicity and antimutagenicity

Several short-term and long-term assays for the assessment of mutagenicity and antimutagenicity of a variety of compounds involving microbial, viral, plant cell and cell lines as well as animal systems have been developed. The short span of the life cycle and available information on genomes, mutation and recombination processes make several viruses, bacteria (E. coli, Bacillus, Salmonella typhimurium), yeast (Saccharomyces cerevisiae), plant cell (Allium cepa, Vicia sativa), plants and animal cell cultures as suitable systems for studying mutagenesis and antimutagenesis (Zhang and Otha, 1993). Amongst all, the assay for mutagenicity testing developed by Maron and Ames, 1983; employing Salmonella typhimurium has been extensively used in the identification of mutagenic and antimutagenic effects of variety of physical, chemical and natural compounds include plant extracts. The Salmonella typhimurium TA97a, TA98, TA100, TA102, TA104, TA1535, 1537, 1538 and some other mutant strains have been commonly employed in the mutagen and antimutagens screening programme (Ames et al., 1975; Maron and Ames, 1983). To make the system more
meaningful, the metabolic activation step has been included to mimic the biotransformation that can occur in animals when chemicals are ingested.

In this assay all the *Salmonella typhimurium* strains are histidine negative while *E. coli* strain is tryptophan dependent. Revertants are identified as colonies that grow in low levels of histidine or tryptophan. Frameshift and base pair substitution defects are represented to identify both types. Additional genetic markers serve to make the strains more sensitive to certain types of mutagens. A list of these additional genetic markers and strain characteristics are shown in Table M1.

The DNA repair mutation (uvrA/B) eliminates excision repair, a repair pathway for DNA damage from UV light and certain mutagens. The presence of the uvrA/B mutation makes the strains more sensitive to the test samples that induce damage in this manner. The uvrA/B mutation is part of a deletion mutation extending into a gene for biotin synthesis; therefore, the biotin requirement is a result of the deletion of this region. The uvrA/B mutation is indicated by sensitivity to UV light.

The rfa mutation changes the properties of the bacterial cell wall and results in the partial loss of the lipopolysaccharide (LPS) barrier increasing permeability of cells to certain types of chemicals (Maron and Ames, 1983).

Although there is considerable development of other methods for the genotoxicity testing of chemicals as some of the genotoxic mechanism would not be detected by a nutritionally reversion assays such as *Salmonella* His" reversion test. In particular chromosomal interchanges, DNA strand breaks and larger chromosome deletion are not efficiently detected in the Ames assay. Thus, other *in vitro* and *in vivo* tests have been recommended for genotoxic assessment of chemicals, which includes *in vitro* micronucleus test, *Saccharomyces cerevisiae* and *Vibrio harveyi* systems (Miadokova *et al.*, 2002; Podgorska *et al.*, 2005).

The mechanisms for inhibition of mutagenesis by plant products are illustrated in Table R3 (De Flora, 1998).
Table R3 Antimutagenic mode of action of plant extracts/ phytocompounds

I. Extracellular mechanisms
A. Inhibiting the uptake of mutagens or precursors by:
   1. Hindering their penetration into
      1.1 the organism
         - body shielding, washing, dietary calcium
      1.2 the cells
         - fatty acid, putrescin, aromatic amino acid
   2. Removal from the organism
      - Dietary fibres
B. Inhibiting endogenous formation of mutagens and carcinogens by:
   1. Inhibiting nitrosation
      - Vitamins (ascorbic acid, α-tocopherol) phenols, food extracts and beverages
   2. Modifying microsomal intestinal flora
      - fermented products
C. Complexation, dilution and/or deactivation of mutagens/carcinogens:
   1. Physical or mechanical means
      - maintenance of physiological pH, dietary fibres
   2. Chemical reaction
   3. Enzyme-catalyzed reaction
   D. Favoring absorption of protective agents
      - Vitamin D3 and analogues

II. Inhibition of mutagens and cancer initiation by cellular mechanisms
A. Modulation of metabolism by:
   1. Inhibiting cell replication
   2. Sequestering mutagens in non-target cells
   3. Inhibiting activation of promutagens
   4. Inducing detoxification
      - phenols, thiols
B. Blocking reactive molecule by:
   1. Reacting with electrophiles
   2. Antioxidant activity and scavenging of reactive oxygen species
   3. Protecting nucleophilic sites of DNA
      - ellagic acid, retinoids
C. Modulation of DNA replication/repair by:
   1. Increasing fidelity of DNA replication
   2. Increasing repair of DNA damage
      - cinnamaldehyde, coumarins, umbelliferone, vanillin, thiols, tannic acid
   3. Inhibiting error-prone repair
      - protease inhibitors
**III. Inhibition of tumor promotion**

1. Inhibition of genotoxic effects
   - Extracellular mechanisms and inhibition of mutagens and cancer initiation by cellular mechanisms

2. Antioxidant activity and scavenging of free radicals
   - Antioxidant activity and scavenging of reactive oxygen species

3. Inhibition of cell differentiation
   - Retinoids, calcium

4. Inhibition of cell apoptosis
   - Retinoids, flavonoids

5. Protection of intercellular communications
   - β-carotene, vitamin A, retinoids

6. Signal transduction modulation
   - Retinoids, flavonoids, D-Limonene

**IV. Inhibition of tumor progression**

1. Inhibition of genotoxic effects
   - Extracellular mechanisms and inhibition of mutagens and cancer initiation by cellular mechanisms

2. Antioxidant activity and scavenging of free radicals
   - Antioxidant activity and scavenging of reactive oxygen species

3. Signal transduction modulation
   - Retinoids, flavonoids, D-Limonene

4. Effect on the immune system
   - α-tocopherol, retinoids, vitamin B12

5. Inhibition of neovascularization
   - Retinoids, thiols

**V. Inhibition of invasion and metastasis**

1. Inhibition of proteases involved on basement membrane degradation and modulation of the interaction with the extracellular matrix
   - Retinoids, protease inhibitors, polyphenols

2. Induction of cell differentiation
   - Retinoids

3. Antioxidant activity
   - Antioxidant activity and scavenging of reactive oxygen species

4. Signal transduction modulation
   - Retinoids, flavonoids, D-Limonene

*Adapted from (De Flora, 1998)*

### 2.4.2. Mutagenicity of plant extracts and phytocompounds

Research on the plants used in folk medicines in the form of beverage and other formulations, and their specific potential efficacy, safety and toxicity has been the subject of intensive investigation. Specific attention is focused on mutagenicity of
plant extracts, herbal formulations and specific phytocompounds. Considerable amount of data have been generated on medicinal and edible plants. In few cases mutagenic compounds have been postulated or identified.

Many reports has been published on screening of Brazilian plants for mutagenicity including the extracts of Achyrocline satureoids, Baccharis amomola, Luchea divaricata, Myricaria tenella, Similax compestris, tripodanthus acutifolius, Cassia corymbosa, Campomanesia xanthocarpa in Ames Salmonella assay with or without S9 and using SOS spot chromatotest microscreen phage induction assay (Vargas et al., 1991; De Saferreira et al., 1999; Fernandes and Vargas, 2003). It has been suggested that the mutagenicity might be due to flavonoids, tannins and anthraquinones, quercetin and caffeic acid. Schimmer and workers (1994) evaluated fifty-five commercial phyto-pharmaceuticals (extract and tinctures) from 44 plant species. The extracts of the plants viz. Alchemillae, Centaurii, Hippcastani, Myrtilli, Hyprici, Trifolii fimbrini showed sign of mutagenicity in TA98 and TA100 Salmonella strains with S9. Sandnes et al. (1992) reported mutagenic potential of extracts of Senna folium and Senna fructus in TA98 strain with S9 in Salmonella test.

Rubiolo et al. (1992) evaluated the mutagenicity of a series of pyrrolizidine alkaloids and extracts of several Italian Senecio species containing pyrrolizidine alkaloids including S. inaeuguidens, S. fuchii and S. cacaliastes. The mutagenicity of extract from Ruta graveolens in Salmonella tester strains TA98 and TA100 has been showed in the presence and absence of S9 mix due to the presence of fluoroquinoline alkaloids (Paulini et al., 1987). Medicinal herbs from the Poland viz. Erigeron canadensis, Anthyllis vulnararia, and Pyrola chloranta have been used for isolation of quercetin, rhamnetin, isoamnetin, apigenin and luteolin flavonoids. Of the above flavonoids only quercetin and rhamnetin revealed mutagenic activity in the test using TA97a, TA98, TA100 and TA102 tester strains Salmonella typhimurium. The presence of S9 rat liver microsome fraction markedly enhances the mutagenic activity of quercetin. Rhamnetin appeared to be much weaker mutagen in the Ames test (Czeczot et al., 1990). Moreover, the aqueous extracts of the plants viz. Lannea edulis and Monots glaber used in traditional medical practice of Zimbabwe and other parts of Africa also showed sign of mutagenicity in TA97a, TA98 and TA100 Salmonella
typhimurium tester strains (Sohni et al., 1995). Mutagenicity testing of the plant essential oils and their monoterpenoid constituents like citral, citronellol (+/-), camphor compound, 1,8-cineole (eucalyptol), terpineol and C-1-menthol revealed that terpineol to be mutagenic in TA102 tester strains both in the presence and absence of S9 mix. Other monoterpenoids have been reported to be non-mutagenic in TA97a, TA98, TA100 and TA102 tester strains in Ames test (Gomes-Carneiro et al., 1998).

Reid et al. (2006) screened the dichloromethane and 90% methanol extracts of 42 South African plants for mutagenicity and antimutagenicity using the Salmonella/microsome mutagenicity assay (Ames) against Salmonella typhimurium TA98 and TA100 bacterial strains in the presence and absence of metabolic activation using S9. The methanol extracts from whole plants of Helichrysum simillimum, Helichrysum herbaceum and Helichrysum rugulosum indicated mutagenicity. (Khader et al., 2007) evaluated the in vitro toxicological properties and potential antimutagenic effects of aqueous extracts of the three plants viz. Nigella sativa, Teucrium polium and Trigonella foenum-graecum. The extracts were tested in primary rat hepatocyte cultures against N-methyl-N'-nitro-N-nitrosoguanidine and applied before, during and after application of MNNG to discriminate between different mechanisms of action. The three plant extracts themselves significantly increased the frequency of chromosomal aberrations. Results suggested that aqueous extracts of the three herbs was neither cytoprotective nor antimutagenic, instead there was an evidence for a mutagenic potential.

2.4.3. Antimutagenic properties of edible and medicinal plants

Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for cancer prevention and have no undesirable xenobiotic effects on living organisms (Ferguson, 1994; De Flora, 1998). Encouraging reports on antimutagenic properties of edible plants have led to increase interest in search of natural phytoantimutagens from medicinal plants from different parts of world; an extensive literature survey on phytoantimutagens has been made and presented in Table R4. Edible plants with antimutagenic activity and chemopreventive potential have been documented from several plants groups like vegetables (Solanum melongena (fruit), Raphanus sativus (root), Allium sativum (bulb), Allium cepa (bulb),
Brassica oleraceae (curds), lycopersicon esculentum (fruit) and spices like Zingiber officinale (rhizome), Syzygium aromaticum (bud), Curcuma domestica, Cuminum cyminum, Carum carvi (seeds), Coriandrum sativum (seed), Piper nigrum (seed) and Cinnamomum zeylanicum (Nakamura et al., 1996; Sangwan et al., 1998; Cortés-Eslava et al., 2004; Mazaki et al., 2006; Jayaprakasha et al., 2007; Shukla and Singh, 2007). However, some interesting reports and recent literature are also reviewed below:

Previously, various studies have indicated that propolis and some of its components, such as the caffeic acid phenyl esters and artepellin C, have antimutagenic and anticarcinogenic effects (Rao et al., 1992; Frenkel et al., 1993). Later, Varanda and workers (1999) showed the inhibitory effect of a propolis extract on daunomycin, benzo(a)pyrene and aflatoxin B1 induced mutagenicity in the Salmonella microsome assay. Another edible food with proven nutritional and therapeutic values throughout the world since ancient times include mushrooms (Chang, 1996). The activities of various mushroom extracts include anticarcinogenic effects (Fullerton et al., 2000; Sliva, 2003), antimutagenic effects (Shon and Nam, 2001; Lakshmi et al., 2003) and protection from blocks to gap junction-based intercellular communication (Cho et al., 2002).

Likewise, four Nigerian common vegetables extracts (B. pinnatum, Dialium guincense, O. gratissium and Vernonia amygdalina) showed antimutagenic effect against reverse mutation induced by EMS and 4 nitro-phenylenediamine and 2-amino fluorine (Obaseki-Ebor et al., 1993). In addition several others plants like C. arabica, C. sinensis, Glycyrrhiza glabra and Eucommia ulmoides exhibit antimutagenic properties (Zani et al., 1993; Nehlig and Debry, 1994; Gupta et al., 2002). Yoshikawa and workers (1996) investigated the antimutagenic effects of specific components of the extracts from eggplant fruits using the Salmonella/microsome assay. The eggplant fruit juice exhibited an antimutagenic activity against 3-amino-1-methyl-5H-pyrido [4, 3-6] indole (Trp-P-2) induced mutagenicity.
Table R4  Antimutagenicity of medicinal and edible plants

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of plant/ Family</th>
<th>Active extracts/ isolated phytocompounds</th>
<th>Active against mutagen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Allium cepa</em> (Liliaceae)</td>
<td>Ethyl acetate extract</td>
<td>IQ, MNNG</td>
<td>Shon <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>3.</td>
<td><em>Aloe vera</em> (Liliaceae)</td>
<td>Di (2- ethyl hexyl) phthalate</td>
<td>2AF</td>
<td>Lee <em>et al.</em>, 2000</td>
</tr>
</tbody>
</table>
| 5.     | *Azadirachta indica* (Meliaceae) | Flavonoid, baicalein Methanol extract of flower/ flavonones | Trp-P-1, Heterocyclic amines, Trp-P-1 | Nakahara *et al.*, 2001;
|        |                        |                                          |                        | Nakahara *et al.*, 2002 |
| 6.     | Berry (Straw, Blue and Ras) | Hydrolyzed tannin containing extract    | MMS, B(a)P             | Smith *et al.*, 2004 |
|        |                        |                                          |                        | Dashwood and Guo, 1993;
<p>|        |                        |                                          |                        | Nakamura <em>et al.</em>, 1996 |
| 8.     | <em>Bryophyllum pinnatum</em> (Crassulaceae) | Ethylacetate and petroleum ether fraction | 4 nitro-o- phenylene-diamine,2 AF, EMS | Obaseki-Ebora <em>et al.</em>, 1993 |
| 13.    | <em>Capsicum annuum</em> | Capsaicin                                | Cyclophosphamide       | Pelkonen and Nebert, |</p>
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<th>14. <em>Castela texana</em> (Solanaceae)</th>
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*Partially adapted from (Sangwan *et al.*, 1998; Musarrat *et al.*, 2006)

**Abbreviations used:**
2 AF, 2-aminoflourene; 2 AAF, 2-acetyl aminoflourene; 2 AN, 2-amino anthracene; 4-NQO, 4-nitroquiniline-N-oxide; AFB1, Aflatoxin B1; B(a)P, benzo (a) pyrene; DMBA, 7, 12, dimethyl benzo(a) anthracene; EMS, Ethyl methanesulfonate; Glu-P-1, 2-amino-6-methylpyrido (1,2-a: 3, 2-d)-imidazole; IQ, 2-amino-3-methyl-imidazo (4,5-f) quinoline; MMS, methyl methanesulfonate; MNNG, N-methyl-N'-nitro-N-nitroguanidine; MNU, N-methyl-N-nitrosourea; NNK, Nitrosamine-4- (methyl nitrosamino), NOP, 4-nitro-o-phenylenediamine; NPD, 4-nitro, 1,2-phenylenediamine; Trp-P1, 3-amino-1, 4-dimethyl-5H-pyrido (4,3-b) indole; Trp-P-2, 3-amino-1-methyl-5H-pyrido (4,3-b) indole.
A fraction isolated from *Terminalia arjuna* was studied for its antimutagenic effect against 4 nitro-o-phenylenediamine (NPD) in TA98 and TA100 tester strains of *Salmonella typhimurium* using the Ames assay. The fraction inhibited the mutagenicity of 2-AF very significantly in both strains while the revertant colonies induced by NPD and sodium azide were reduced moderately. $^1$H-NMR, $^{13}$C-NMR, IR and UV spectroscopic data of the fraction revealed tannins as active constituents (Kaur et al., 2000).

The effects of sesamol, a phenolic compound responsible for the high resistance of sesame oil to oxidative deterioration as compared with other vegetable oils, have been investigated after mutagen treatment in various strains of *Salmonella typhimurium*. Sesamol was shown to exhibit strong antimutagenic effects in the Ames tester strains TA100 and TA102 (Kaur and Saini, 2000).

Antimutagenic properties of South African herbal teas were investigated by (Marnewick et al., 2000). An aqueous extracts of fermented and unfermented rooibos tea (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*) showed antimutagenic activity against 2-acetylaminofluorene (2-AAF) and aflatoxin B$_1$ (AFB$_1$)-induced mutagenesis using TA98 and TA100 tester strains in the presence of metabolic activation.

Nakahara et al. (2001) have shown that a methanolic extract of *Oroxylum indicum* strongly inhibit the mutagenicity of Trp-P-1 by Ames test. Later, Nakahara and workers (2002) identified the major antimutagenic constituent as baicalein with an IC$_{50}$ value of 2.78 ± 0.1 μM. The potent antimutagenicity of the extract has been correlated with the high content of baicalein, which also act as a desmutagen and inhibit the N-hydroxylation of Trp-P-2.

Similarly, Pillai et al. (2001) studied that a number of antimutagenic agents, e.g. green tea catechins, and other antioxidants are able to suppress the emergence of resistance. In many cases, these agents are capable of exerting these effects at doses which by themselves produce no visible effect on growth.

Ferrer et al. (2002) demonstrated the antimutagenicity of *Phyllanthus orbicularis* against hydrogen peroxide using *Salmonella* assay. Likewise, Miadokova and workers
(2002) evaluated the potential antimutagenic effect of the plant extract of *Muscari racemosum* bulbs, rich in 3 benzylidene-4- chromanones on three genetic model organisms. The mixture of three homoisoflavonoids have been tested together with diagnostic mutagens in the Ames assay on four bacterial strains *Salmonella typhimurium* TA97, TA98, TA100, TA102 in the toxicity and mutagenicity/antimutagenicity assay on the yeast strain *Saccharomyces cerevisiae* D7, and in the simultaneous phytotoxicity and clastogenicity/ anticlastogenicity assay on *Vicia sativa*. The extract exerted antimutagenic and anticlastogenic effects due to the presence of homoisoflavonoids, which may be included in the group of natural antimutagens. This genotoxicological study suggested that homoisoflavonoids from *M. racemosum* owing to antimutagenic and anticlastogenic properties are of great pharmacological importance, and might be beneficial for prevention of cancer.

Pasquini et al. (2002) determined the antimutagenic potential of chloroform, acetone, methanol, acidified methanol, diethyl ether and ethyl acetate extracts of *Terminalia arjuna* (bark) against the model mutagen 4-nitroquinoline-N-Oxide (4 NQO) using the *Salmonella/microsome*, comet, and micronucleus (MN) tests. The 4-NQO mutagenicity was inhibited by more than 70% in the *Salmonella/microsome* test at the highest nontoxic extract dose of ethyl acetate (50 µg/plate), chloroform (100 µg/plate), acetone, (100 µg/plate), and methanol (500 µg/plate). A less marked antimutagenicity activity (inhibition of about 40-45%) was observed for the acidic methanol and diethyl ether extracts. The comet assay showed that acetone extract (100 µg/ml) was more effective in reducing the DNA damage caused by 4-NQO (ca. 90%), whereas the chloroform, ethyl acetate, and diethyl ether extracts were cytotoxic. In the MN test, the decrease in 4-NQO clastogenicity was observed by testing the mutagen especially with chloroform and ethyl acetate extracts (inhibition about 40-45%). The acetone and methanol extracts showed a less marked activity (33% and 37%, respectively).

Avila et al. (2003) studied the antigenotoxic, antimutagenic and ROS scavenging activities of a *Rhoeo discolor* ethanolic extract. Its mutagenic capacity was investigated by the Ames test and genotoxic activity in primary liver cell cultures by using the unscheduled DNA synthesis assay. This extract was not mutagenic when tested with *Salmonella typhimurium* strains TA97a, TA98 and TA100, and it did not
elicit unscheduled DNA synthesis in hepatocyte cultures. In addition, the results showed that Rhoeo extract is antimitagenic for S. typhimurium strain TA102 pretreated with ROS-generating mutagen norfloxacin in the Ames test, and protects liver cell cultures against diethylnitrosamine induction of unscheduled DNA synthesis even at 1.9 ng per dish, which was the lowest dose tested. Also, Rhoeo extract showed less radical scavenging effect than quercetin, but similar to that of a-tocopherol and more than ascorbic acid, respectively.

In the same year, the antimitagenic, comutagenic and cytotoxic effects of saffron and its main ingredients were assessed using Ames Salmonella test system. The saffron component responsible for this unusual co-mutagenic effect is safranal. In vitro colony formation test system, saffron exhibits a dose dependent inhibitory effect only against human malignant cells (Abdullaev et al., 2003).

While in search for novel polyphenolic antimitagenic agents from Indian medicinal plants, Kaur et al. (2003) examined the water, acetone, and chloroform extracts of Terminalia bellerica for their antimitagenic potency using the Ames Salmonella/microsome assay. Acetone extract exhibited variable inhibitory activity of 65.6%, and 69.7% with 4-O-nitro phenylenediamine (NPD) and sodium azide respectively (as direct acting mutagens), and 81.4% with 2-amino flourene (2-AF) an S9-dependent mutagen.

Hamss et al. (2003) showed that bell pepper (Capsicum annuum) was effective in reducing the mutational events induced by ethyl carbamate and MMS and black pepper (Piper nigrum) was only effective against ethyl carbamate. Suppression of metabolic activation or interaction with the active groups of mutagens could be mechanisms by which the spices exert their antimitagenic action. In the same year Miyazawa and Hisama, isolated the phenylpropanoids from the buds of clove (Syzygium aromaticum) that possess antimitagenic activity. The isolated compounds suppressed the expression of theumu gene following the induction of SOS response in the Salmonella typhimurium TA1535/pSK1002 that have been treated with various mutagens.

Shon et al. (2004) assessed the antioxidant and antimitagenic activities of red, yellow and white onion extracts. The study demonstrated that the antimitagenicities and
The antioxidant properties of ethyl acetate extract against mutagens were related to their phenols and flavonoids, which are heat stable and losses digestive juices are relatively low. Similarly, Smith et al. (2004) showed that the fresh juices and organic solvent extracts from the fruits of strawberry, blueberry, and raspberry inhibit the production of mutations by the direct acting mutagen methyl methanosulfonate and the metabolically activated carcinogen benzo(a)pyrene.

The antimutagenic activity of coriander juice against the mutagenic activity of 4-nitro-o-phenylenediamine, m-phenylenediamine and 2-aminofluorene was investigated using the Ames reversion mutagenicity assay (his$^{-}$ to his$^{+}$) with the S. typhimurium TA98 strain as indicator organism. Aqueous coriander extract significantly decreased the mutagenicity of metabolized aromatic amines (AA) in the following order: 2-AF (92.43%) > m-PDA (87.14%) > NOP (83.21%). The chlorophyll content in vegetable juice was monitored and its concentration showed a positive correlation with the detected antimutagenic effect (Cortés-Eslava et al., 2004).

In the year 2005, Geetha and workers evaluated the antimutagenic activity of quercetin, ascorbic acid and their combination against an oxidative mutagen, t-butyl hydro peroxide. In vitro antioxidant scavenging activity was tested for DPPH free radical, superoxide anion, hydrogen peroxide and hydroxyl radical in their specific systems. Quercetin (0.5-8 nmol/plate) and ascorbic acid (0.1-100 μmol/plate) showed significant effect. Moreover, the antimutagenic activity of Sesbania javanica, an edible vegetable flower DMSO extract against aflatoxin B1 (AFB1) and benzo(a)pyrene (B(a)P), was also demonstrated using the Ames test (Tangvarasittichai et al., 2005).

The antimutagenic potential of caffeic, ferulic and cichoric acids extracted from plant species of genera Echinacea (L) Moench, as well as of phenolic acids in the plate-incorporation test was also described against 3-(5-nitro-2-furyl) acrylic acid (5NFAA) and sodium azide mutagenicity (Birosová et al., 2005).

Rosa et al. (2006) studied the chemical composition, an in vivo antioxidant properties and mutagenic/antimutagenic effects of methanolic extracts of Hibiscus tiliaceus flowers. The extract was not mutagenic in either Salmonella typhimurium or S.
cerevisiae and showed a significant antimutagenic action against oxidative mutagens in S. cerevisiae. Likewise, Nogueira et al. (2006) investigated the Melampodium divaricatum flower extract for mutagenic and antimutagenic effect in the Salmonella/microsome assay. The tested extract was not mutagenic in the strains TA100, TA98, TA97a and TA102 and decreased the mutagenicity of aflatoxin B1, benzo(a)pyrene and daunomycin. In the same year, Thériault and workers showed good antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products.

Cariño-Cortés et al. (2007) studied the antimutagenic effect of methanolic extracts obtained from leaves, root, and flowers of the Stevia pilosa and Stevia eupatoria using the Ames test with and without metabolic activation. The best antimutagenic effect was obtained with leaves of both species and the flowers of S. eupatoria (99%).

Similarly, Mezzoug et al. (2007) reported the chemical composition of Origanum compactum essential oil and its mutagenic and antimutagenic activities by the somatic mutation and recombination test (SMART) in Drosophila melanogaster. Moreover, Lazarou et al. (2007) assessed that a polyphenolic mixture derived from sesame-seed perisperm (SSP) strongly reduced the mutagenicity of hydrogen peroxide, sodium azide, and benzo(a)pyrene in Salmonella typhimurium TA98 and TA100 strains.

In the same year, Jayaprakasha and his colleagues investigated the antioxidant and antimutagenic activities of Cinnamomum zeylanicum fruit extracts. The antioxidant activity of the extracts was found in the order of water>methanol>acetone>ethyl acetate. The antimutagenicity of water extract was followed by acetone, methanol and ethyl acetate. The results of the present study indicated that under-utilized and unconventional part of cinnamon is a good source of antioxidant and antimutagenic phenolics.

In a study conducted in our laboratory, methanolic extracts of Acorus calamus (Rhizome), Hemidesmus indicus (Stem), Holarrhena antidysenterica (Bark) and Plumbago zeylanica (Root), showed no sign of mutagenicity to Salmonella typhimurium tester strains at tested concentrations (25 to 100 µg/ml). However, all the extracts exhibited significant dose-dependent antimutagenicity from 18.51 to 82.66% against NaN3 and MMS induced mutagenicity (reference). Similarly, the mutagenic
and antimutagenic activities of hexane, chloroform, ethyl acetate and methanol extracts from leaves of *Myrtus communis* were investigated by the *Salmonella typhimurium* assay. The different extracts showed no mutagenicity when tested in TA98 and TA100 either with or without metabolic activation (S9). On the other hand, each of the tested extracts exhibited a significant protective effect against the mutagenicity induced by aflatoxin B1 (AFB1) in *Salmonella typhimurium* TA100 and TA98, and against the mutagenicity induced by sodium azide in TA100 and TA1535 assay system (Hayder *et al.*, 2008).

Bourgou *et al.* (2008) assayed the methanolic extracts from shoots and roots of Tunisian *Nigella sativa* for their antioxidant and antimutagenic activities shoots and roots showed comparable and strong superoxide scavenger activity. However, shoot extract exhibited higher DPPH radical scavenging, reducing and chelating activities than root extract. When tested for mutagenic and antimutagenic activities by Ames test, root extract exhibited stronger activity than shoot extract with an inhibition percentage of 71.32%.

In the same year, Kokotkiewicz *et al.* (2009) reviewed Honeybush (*Cyclopia* sp.), as a rich source of compounds with high antimutagenic properties. On the other hand, Di Sotto *et al.* (2009) studied the effects of 3,4-secoisopimar-4(18),7,15-trien-3-oic acid, a diterpenoid isolated from *Salvia cinnabarina*, in the Ames test on *Salmonella typhimurium* TA98 and TA100 and on *Escherichia coli* WP2uvrA, with and without metabolic activation.

Alizarin (1,2-dihydroxyanthraquinone), a dye was isolated and characterized from *R. cordifolia* and evaluated for its antigenotoxic potential against a battery of mutagens viz. 4-nitro-o-phenylenediamine (NPD) and 2-aminofluorene (2-AF) in Ames assay using TA98 tester strain of *Salmonella typhimurium*; hydrogen peroxide (H2O2) and 4-nitroquinoline-1-oxide (4NQO) in SOS chromotest using PQ37 strain of *Escherichia coli* and in comet assay using human blood lymphocytes (Kaur *et al.*, 2010).

Parvathy *et al.* (2010) studied the synthesis, antioxidant and antimutagenic attributes of Curcumin–amino acid conjugates. Curcumin-β-diglucoside (III), prepared by glycosylation of curcumin (I) at the phenolic hydroxyl group, was soluble in water at
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10 mg/ml concentration. Studies of the radical-scavenging, as well as antioxidant properties, of Curcumin-β-diglucoside III at different concentrations showed that these activities were higher than that of curcumin I. The mutagenicity studies showed that curcumin I, as well as Curcumin-β-diglucoside III, afforded high protection against the mutagenicity of sodium azide to *Salmonella typhimurium* TA1531 and TA98. Also, Curcumin-β-diglucoside III exhibited higher antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* but showed lower activity against *Bacillus cereus* and *Yersinia enterocolitica* than did curcumin I. The results clearly demonstrate that conjugation of the phenolic hydroxyl group of curcumin to a sugar moiety rendered it water-soluble whilst retaining/enhancing its *in vitro* antioxidant, antimutagenic and antibacterial properties.

Similarly, Khader *et al.* (2010) demonstrated the antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. The results of this investigation clearly indicated an inhibitory effect of the plant extracts on MNNG mutagenicity, while the extracts had no effect on cytotoxicity indicators such as necrosis and apoptosis. The effects obtained can be attributed to a direct antimutagenic activity and an increased recovery at the chromosomal level.

2.4.4. Major group of phytocompounds and their antimutagenic potentials

Extensive research in the last few decades on detection and characterization of antimutagenic compounds from edible, non-edible and medicinal plants/herbs has demonstrated a great diversity. Several authors have suggested that phyto-antimutagens may belong to any of the following major class of phytocompounds. Major emphasis has been laid on the flavonoids, phenolics, coumarins, anthraquinone, tannins, terpenoids, diterpenes and several others as specified in Table R4. More than 500 compounds belonging to at least 25 chemical classes have been recognized as antimutagenic (Boone *et al.*, 1990). In the recent years, there has been an increased interest in identifying the antimutagenic and anticarcinogenic constituents of both dietary and medicinal plants all over the world. The major classes of antimutagenic compounds are briefly described below:

**Flavonoids:** Flavonoids are polyphenolic compounds ubiquitously present in plants. More than 4000 different flavonoids have been isolated and identified so far. This
class of phytochemicals received greater attention as they possess several biological activities including antimutagenic and anticancer (Harborne and Baxter, 1995). Some common flavonoids are glabrene (isoflavone), quercetin, myricetin, kaempferol, fisetin, morin and hesperetin (Park et al., 2004; Geetha et al., 2005). Snijman et al. (2007) compared the antimutagenic properties of the most prevalent flavonoids in rooibos (Aspalathus linearis) in the Ames test using tester strains TA98 and TA100 against 2-acetamido-fluorene (2-AAF) and aflatoxin B1 (AFB1) as mutagens. The protective activity was possibly related to properties described for flavonoids and/or tannins acting as potential inactivators of enzymes involved in the mutagen metabolism. Likewise, Loh and workers (2009) aimed to determine the level of quercetin in the aqueous and methanol plant extracts for the investigation of mutagenic effects of quercetin in the Ames test using TA98 and TA100 strains. Both the extracts demonstrated antimutagenic properties at concentrations up to 100 µg/ml in the absence and presence metabolic activation. Moreover, in a recent report Wozniak et al., 2010 showed the antimutagenic activity of isoflavonoid fractions obtained from methanolic extract of Belamcanda chinensis rhizomes by the Ames test. The fractions enriched in isoflavonoids, inhibited indirect mutagenesis in TA98 almost completely.

Phenolic compounds: Phenolic compounds are most widely studied groups of compounds from natural food and medicinal plants and also implicated for different biological activities. Certain phenolic compounds like ellagic acid found in strawberries, raspberries, grapes, and walnut etc have been found to be antimutagenic (Lansky and Newman, 2007). The compounds like epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechins (EGC), (-) epigallocatechin gallate (EGCG) of green tea and black tea have also been reported to exert antimutagenic activity (Kuroda and Hara, 1999; Ohe et al., 2001; Geetha et al., 2004).

Coumarins: Coumarins are 2H-1-benzopyran-2-ones, widely distributed in the vegetable and other plants. A wide range of structure with varying complexity occurs in angiosperms. Coumarins have been shown to behave both as antimutagens as well as anticarcinogens (Harborne and Baxter, 1995; Okamoto et al., 2005). For instance, the umbelliferone, 8-methoxysoralin, imperatorin, osthol have been described with antimutagenic activity. The antimutagenic activity of a wide array of phytochemicals
including anthraquinones isolated from *Cassia occidentalis* was also demonstrated by Yadav *et al.* (2010).

**Diterpenoid:** Diterpenoid like secoisopimarane isolated from *Salvia cinnabarina* M. demonstrated antimutagenic activity (Di Sotto *et al.*, 2009). Four novel dibenzoate diterpenes, pulcherrimins A, B, C and D obtained from roots of *Caesalpinia pulcherrima* were found active in DNA repair deficient yeast mutant (Patil *et al.*, 1997). Eugenol, active constituent in clove oil has been reported to possess significant antimutagenic activity (Ramos *et al.*, 2003). Similarly, antiproliferative, apoptotic and antimutagenic activity of isolated compounds from *Polyalthia cerasoides* seeds were studied by Ravikumar *et al.* (2010). Phytochemical investigation of the petroleum ether fraction of *Polyalthia cerasoides* seeds led to the isolation of two phytosterols (α-spinasterol and spinasterol) and a clerodane diterpenoid. The isolated compounds possessed antiproliferative and antimutagenic properties. Furthermore, the antimutagenic effects of some terpenes viz. linalool, linalyl acetate and β-caryophyllene have also been described (Di Sotto *et al.*, 2008).

**Organosulfur compounds:** Ajoene and allicin have been found in garlic extract with significant antimutagenic activity (Ishikawa *et al.*, 1996). Various other organosulfur phytocompounds like caffeine, trigonelline and piperine have been demonstrated to possess antimutagenic properties (Wu *et al.*, 2007; Vellaichamy *et al.*, 2009).

**Alkaloids:** Antioxidant and antimutagenic properties of the monoterpene indole alkaloid psychollatine and the crude foliar extract of *Psychotria umbellata* were studied by (Fragoso *et al.*, 2008). Psychollatine and the crude foliar extract of *P. umbellata* showed protective effect against oxidative stress in yeast, acting both as antioxidant and antimutagenic agents.