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Bioprospecting for drug research and functional foods for the prevention of diseases—Role of flavonoids in drug development

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Bioprospecting is the exploitation of wild diversity (natural bioresources) in search for useful resources such as medicines. Research in the area of chemistry of natural products when coupled with sources of local traditional medicines and aided by pharmacological and antimicrobial studies may lead to the development of newer and effective drugs. Studies of the functional foods such as broccoli, garlic, citrus fruits, onions, oat, soy, tea, etc. may help in the prevention of diseases such as cancer, coronary heart diseases, inflammation, allergy, etc. The pharmacological activities of flavonoids are gaining importance with newer findings on the beneficial health effects. Flavonoids possess radical scavenging properties. There is a wide scope for integrating natural products based pharmaceutical industries with fuel and energy industries dealing with petrocrops, biodiesel, etc. Convenient synthesis of natural products may pave the way for the scale up and process development engineering.

Keywords: Bioresources, Drugs, Flavonoids, Functional foods, Medicinal plants, Natural products

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Introduction

Research on drugs for different diseases is being carried out world over. Bioinformatics has also joined this race. Fight for survival of the mankind on this earth is going on from the time immemorial. Allopathic system of medicines has brought a great hope to the mankind by eliminating several diseases. However, still there are several diseases, which are offering challenge to the medical scientists. While some diseases get eliminated, some more appear in some corner of the world. There is a growing need for finding out newer and effective designer molecules, which can fight the microbes and other abnormal metabolic disorders in human system causing the diseases and repair the system.

Several drugs and medicines (pharmaceuticals) in current use are derived from natural resources. In the USA alone[1], a total of about 40 percent pharmaceuticals are derived from wild species as follows: Plants, 25; Microorganisms, 13; and, animals, 3%. Wilson[1] reported that nine of the ten leading prescription drugs originally were obtained from organisms. Chloroform extract from the leaves of *Thymus satureioides* Coss (Labiatae) shows anti-inflammatory activity attributed to ursolic and oleanolic acids[2]. Methanol extract showed a significant radical-scavenging activity due to flavonoids[2], known as biomolecules.

Antibiotics have been derived mostly from the fungi. There exists a large number of species of different microorganisms such as ascomycetes fungi. Probably less than 10 percent of the world's ascomycete species have been discovered[1]. Thus there exists wide scope still to discover and explore rest ascomycete species. Algal species, *Spirulina platensis*, offers a great promise both as a functional food and possesses anticancer and radical scavenger properties.

The oil from non-edible seeds of trees like *Madhuca longifolia, Azadirachta indica, Pongamia pinnata* may be trans-esterified for use as biodiesel[3-6]. From nearly 400 species[3-6] belonging to six laticiferous families (*Euphorbiaceae, Asclepiadaceae, Moraceae, Apocynaceae, Convolvulaceae, Sapotaceae*), *Calotropis procera, C. gigantea, Euphorbia trigona, E. neriifolia, E. antisyphilitica, E. royleana, E. tirucalli, E. lathyris, Hemidesmus indus, Padilanthus tithymaloides, Argyreia nervosa,* and *Baliospermum montanum,* were identified as potential petrocrops. Latex, resins,

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waxes and other extractives present in these laticiferous and resinous plants contain hydrocarbons, triterpenes etc., which can afford petroleum like fuel on hydrocracking. Most of these plants had been acting as traditional folk medicines[7]. Thus, petrocrops may be studied for medicines as well as liquid fuels.

Bioprospecting for Medicines

Exploration of wild diversity for useful resources is called bioprospecting. This has grown into a respectable and promising industry within a global market eagerly awaiting the discovery of new and effective pharmaceuticals. It also covers the discovery of new petroleum sources (petrocrops), food sources, fibers, materials, etc. including hemicellulosic, cellulosic, starch, lignin, etc. based chemicals[3-6].

Wilson[1] has given following examples for the use of medicinal plants by tribes of upper Amazon: *Abuta grandiflora* (fever, snake bite); dye plant, *Arrabidacea chica* (anaemia, conjunctivitis); monkey ladder, *Bauhinia guianensis* (amoebic dysentery); wild senna, *Sena raticulata* (bacterial infection); wild mango, *Grias nenberthu* (tumors, dysentery); etc.

Fresh leaves[8] of Italian shrub, *Rubus ulmifolius*, are used in local folk medicine against abscesses, furuncles, ulcers, redden eyes, for diarrhea, hemorrhoid, intestinal inflammation etc. In Chilean folk medicines, it is used against hypoglycemic activity. Gallic and ferulic acids present in the methanol extract of the leaves of *R. ulmifolius* possess antimicrobial activities. The algal species[3-6], *Spirulina*, produces proteins, β-carotenes, antiviral compounds such as polysaccharides (spirulans) and anticancer compounds, hydrogen and may absorb toxic metals from wastewater.

Serendipity has also helped in the discovery of several drugs. One example is chance curing of wound, which resulted in the development of sulpha drugs. A chance discovery may also open horizons for the development of other more vital drugs with some variations in the chemical structures, as is known in chemotherapy. Cyclosporin was discovered as a drug for immunosuppression in human, when such a compound was discovered from an obscure fungus[1]. Drugs have been developed from frog poisons, snake venoms, and may be developed from insect poisons, other natural toxins, animals, algae, yeast etc. in an optimum concentration where toxicity to human is under control.

Eucalyptus leaves yield essential oil containing 1, 8-cineole[9], and ursolic acid and have been reported to show anti-inflammatory, antibiotic, antiarthiritic, antiulcer and antilipidemic properties[5,6,9,10]. Hepatoprotective activity of ursolic acid in the rats compared well with that of silymarin [9,10]. *Calophylum longiferum* variety *austocariaceum* contains anti-HIV substance, (+) – calanolide A[1]. *Aloe vera*, which has a number of medicinal properties as pain reliever, anti-inflammatory, anti-allergic, skin healer and healer for bursitis, arthritis, bronchitis, sinusitis, etc, may be studied in detail for pharmacological properties.

Medicinal Properties of Functional Foods

Functional foods are plant-based diets high in fruits and vegetables[11] and can reduce the risk of chronic disease, particularly cancer. Such foods include oat, soy, flaxseed, tomatoes, garlic, broccoli, citrus fruits, tea, cranberry, wine and grapes etc. Tea contains flavonoids such as quercetin, kaemferol, myricetin, apigenin and luteolin, which are now being considered for their pharmacological activites[12]. Soybean isoflavones have advantages against coronary heart disease[11,13]. It contains anticarcinogens which include protease inhibitors, phytosterols, saponins, phenolic acids, phytic acid and isoflavones (genistein, diadzein)[14]. The tomatoes are reported in risk reduction of cancer attributed to lycopene[11].

Garlic (*Allium sativum*) contains organosulfur compounds and possesses cancer chemopreventive, antibiotic, antihypersensitive, and cholesterol lowering properties[11]. Broccoli and other cruciferous vegetables have been studied for anticancer properties[15]. Sulforaphane present in broccoli has received lot of attention[16]. Citrus fruits show health benefits and contain vitamin C, foliate, fiber, limonoids, flavonoids etc[11,17].

Onions (*Allium cepa*) have been studied for prevention of cancer and cardiovascular disorders[18]. Onions contain fructans, flavonoids and organosulfur compounds, which possess anti-inflammatory, anti-allergic, anti-microbial and anti-thrombotic activity[19]. The flavonoids have beneficial health effects activity in reducing risks of coronary heart diseases and different types of cancers[19-23]. Quercetin and kaemferol are the major flavonoids in onion[22,23]. Turmeric (*Curcuma domestica*) and neem (*Azadirachta indica*) have beneficial health effects. Production of β-carotenes, amino acids, vitamin B-12,

GLA essential fatty acids, xanthophylls, sugars and proteins from algal biomass, *Spirulina platensis*, has received lot of attention and polysaccharides may be studied for antimicrobial properties and anti-viral properties against HIV. *S. platensis* also has properties such as strengthening immune system, building blood, radical scavenger and anticancer effects.

Biodiesel, Petroleum and Medicinal Products from Petrocrops and Seed Oils[3-7]

Jatropha curcas seed oil is being studied for its use as a biodiesel and as a lubricant. *Ricinus communis* oil may be used in industry and has medicinal properties. Jojoba (*Simmondsia chinesis*) oil can be used as a lubricant and also found useful in cosmetics. There are other seed oils, which may be studied as biodiesel or biolubricants. *Calotropis procera* yield latex and biocrude. It contains poisonous chemicals and also some pharmaceutically active compounds including compounds having anti-tumour activites. *Lantana camara* and *Tabernaemontana divaricata* also yield biocrude and contain some pharmacologically active compounds. *Eucalyptus lanceolatus* can yield biocrude and is reported to contain pharmaceutical and medicinal oil, industrial oils and perfumery oils. Similarly, *Euphorbia lathyris, Ginkgo biloba, Hydnocarpus wightiana* may also serve as a petrocrop or biodiesel as well as medicines. Thus, such plants may be studied for the possibility of their commercial exploitation to obtain petroleum, lubes, diesel oil, medicines and other value added products.

Antiparasitic Drugs from Natural Products

Plant polyphenols possess antiparasitic property[24]. *Vernonia brachycalyx* (Astraceae)[25] shows antiprotozoal activity attributed to 2 - epicycloisobra chycoumaranone epoxide and its stereoisomer. Lopes *et al*[26] showed the prevention of transmission of chagas disease by blood transfusion using lignans such as grandisin and veraguensin. The activity of these lignans was 40 times higher than the reference drug, gentian violet. The lignans[24,25] from leaves of *Zanthoxyllum naranjilla* (Rutaceae) were tested for their activity against the bloodstream forms of two strains of *I. cruzi in vitro*. Oxidative coupling of lignans with flavonoids yield flavonolignans, another group of flavonoids having medicinal properties.

Phlorizidin, a naturally occurring dihydrochalcone glycoside from *Micromelum tephrocarpusm* (Rutaceae), inhibits the induced permeability in *Plasmodium* infected erythrocytes to various substrates including glucose. The licochalcone A, which was first isolated from *Glycyrrhiza glabra* (Fabacea) is the subject of preclinical studies[24,27]. The anti-parasitic activity has been well documented against parasites such as *P. falciparum, L. donovani and L. major*. A large number of chalcones have been sythesised and their structure-activity (relation) has been determined for their antiplasmodial, antileishmanial and tryptocidal activity[27]. Chalcones can be easily converted to flavonoids chemically.

Flavonoids possess antiplasmodial activity. Methoxylated flavonones, artemetin and casticin, act synergistically[28] with artemisinin against *P. falciparium in vitro*. Sakuranetin (100%) and 7-methoxyaromadendrin (86%) inhibited *I. Cruzi*[29] to show antiprotozoal activity. Specific flavonoids (biomolecules)[30] could effect transport mechanisms in *Leishmania*. *L. tropica* recombinant domain efficiently found different classes of flavonoids with the following affinity : flavone > flavonone > isoflavone > glucorhamnosyl-flavone. A reversing effect was observed with flavones such as dimethyallyl-kaemferide at low concentration or apigenin at higher concentration. Flavonolignans, formed or biosynthesized by the oxidative coupling of lignans and flavonoids, may be studied for antiparasitic properties

Monoterpenes have been reported to constitute antiprotozoal drugs[24]. Sesquiterpenes, germacranolide sesquiterpene lactone and furanoheliangolides[32], were found to have antiplasmodial activities against *Leishmania promastigotes* and *Trypanosoma epismastogotes in vitro*. In fact, the antiprotozoal potential of sesquiterpenes is well established[24, 32]. Parthenin derivatives have been found to be active[24] e.g. in *Leishmania* and *Trypnosoma.* Sesquiterpene lactones, brevilin A and dehydrozaluzanin C, were discovered from plants[24]. Epinstanol, which is isolated from the bark of *Oxandra espinate* (Annonaceae) and synthesized, is active against *Leishmania promastigotes* and *Trypanosoma emastigotes*[31]. Jatrogrossidione and jatrophone, the diterpenes isolated from *Jatropha grassidentata* and *J. isabelli* (Euphorbiacea) respectively, showed significant activity against *Leishmania promastigotes*

in vitro. Several diterpenes (labdane derivatives) have been found to show antiparasitic properties[24,33]. Triterpenes and saponins from plants show antineoplastic, antihelmintic and antiviral activities. The triterpene betulinic acid showed antiplasmodial activity[34]. Steroidial saponin, muzanzagenin from *Asparagus africanus* (Liliaceae) possesses antileishmanial and antiplasmodial activity. Similarly, limonoids and quassinoids also have biological activities[35].

Steroidal alkaloids, α–chaconine and α-solarmargine, possess antipansomal activity[33]. Most alkaloids (cocaine, morphine or semisynthetic LSD, quninine[24,36]) are toxic and have medicinal properties. Quinine extracted from *Cinchona succisubra* (Rubiaceae) is an established drug for malaria[24,36]. The natural quinoline derivatives such as chimanin B was found to have activities against parasites causing cutaneous leishmaniasis. Two piperodino-4-quinolinone alkaloids, dictyolomide A and B isolated from *Dictyoloma incanescens* (syn. *D. vandellianum*) and *D. peruviana* (Rutaceae), showed lysis of various strains of *Leishmania promastigotes*[37]. Quinolines are present not only in plants, but also in lignite and coaltars, the latter also contains carcinogenic polycyclic aromatic compounds.

Pharmacological Properties of Flavonoids and Related Compounds

About 4000 different naturally occurring flavonoids have been reported[38,39]. Flavonoids also exist as O-glycosides, C-glycosides, glucuronosides, sulfates and some have methoxyl groups and may be attached to lignans as well. Flavonoids can inhibit and sometimes induce a large types of enzyme systems[38,39]. Some of these enzymes are involved in important metabolic pathways that regulate cell division and proliferation, platelet aggregation, detoxification, inflammatory, immune response, etc.

Antioxidant properties of flavonoids may protect tissues against oxygen free radicals and lipid peroxidation. Flavonoids may help in the prevention of antherosclerosis, cancer and chronic inflammation and may inhibit ageing[38-40]. These may affect the bioenergetics in metabolic systems and may inhibit several oxidative coupling reactions by scavenging the free radicals present in the metabolic systems. Thus, the studies of the biochemistry of metabolic reactions of flavonoids are important.

In The Netherlands[41], major source of flavonoids are: tea, 40; onion, 29; and, apple, 7%. Studies are needed on the role of flavonoids of common vegetables and fruits in prevention of cancer, coronary heart and neurological diseases.

Bioavailability of flavonoids as aglycones and their absorption in the human system is also important. Release of aglycones from O-glycosides is easier than from C-glycosides. The bioavailability of quercetin from onions and apples was studied and their absorption was also shown up to different extents[42]. Repeated intake of quercetin containing food may result in accumulation of quercetin in blood and may increase the antioxidant capability of blood plasma. Flavonoids showed a protective action towards oxidation of LDL *in vitro*[38-42].

Flavonoids have beneficial effects in cardiovascular diseases, ageing and neurodegenerative disorders[40]. In human metabolism, flavonoids undergo biochemical reactions e.g. deglycosylation resulting metabolism of aglycones such as quercetin, hesperetin, naringenin and epicatechin to glucuronides, sulfates and O-methylated forms during transfer across the small intestines and in liver, colon, etc.[43]. Flavonoids may also get degraded to simple phenolic acids, which may be absorbed and may be metabolised in liver[43].

Flavonoids have the potential to bind to the ATP-binding sites of a large number of proteins. Thus, flavonoids may also affect and modify the bioenergetics of the metabolic pathways, which may thus be biochemically geared for the prevention and treatment of different diseases. Biochemistry of flavonoids and their metabolism with reference to energy dynamics may be studied in detail. Flavonoids may act as the potential inhibitors of protein kinase C tyrosine kinase and PI3-kinase. Most of the studies were based on quercetin. Flavonoids present in peanut skin may also have antioxidant activity and may help in prevention against cardiovascular diseases, Alzheimer disease, cancer inhibition etc.[44]. Antioxidant activity[45, 46] of flavonoids was generally determined by the number and position of hydroxyl groups on the flavonoid structure. The more the –OH groups, the stronger the antioxidant activity. The flavonoids present in peanut skins, green tea and grape seeds have been demonstrated to have multiple human health benefits, such as lower LDL level of serum/liver, inhibition of LDL oxidation thus

preventing cardiovascular diseases, protection of DNA from free radical attack leading to lower the risk of cancer, inhibition of histamine thereby preventing inflammation[44].

Epicatechin contents in Brazilian woods than in Oak suggested an essential role of the flavonoid in the antioxidant activity of these woods[47]. The hydrophilic flavonoids (flavonols, procyanadins and flavone) had the highest interaction with the polar heads of phospholipids at the lipid-water interface of membranes providing prevention against lipid oxidation[48]. Kolavarin, a biflavonoid from seeds of *Garcinia kola,* is eaten as a refreshing pastime and used traditionally in treating laryngitis, cough, mouth infection and liver disorder in some West African countries[49]. Kolavarin shows protective effects against oxidative damage by scavenging both OH and $O_2^{\cdot}$ radicals[49]. All extracts from *Polymia sonchifolia* containing sesquiterpenes, lactones and flavonoids (3,7 di-O-methyl quercetin and 7-O methyl hesperetin) had more scavenging potential than that of *Ginkgo biloba* and rutin[50]. *Ourantee parviflora* (Ochnaceae) is used in Brazil for wound healing and possesses anti-rheumatoid properties[51-53]. The plant contains flavonoids (rutin) and biflavonoids having (apigenin, amenthoflavone, agasthiflavone)[54]. The extracts and species showed free radical scavenging and antioxidant activities.

The lignin isolated from the wastewater of pulp and paper industry was found to show antioxidant activity[55]. The wastewater from pulp and paper industry may be screened for flavonoids and several other polyphenols, terpenoids, steroids, alkaloids, lignans, etc. The pharmacological activities of these components including flavonoids, lignans, flavonolignans, terpenoids, etc. may also be studied, so that the wastewater of black liquor from paper industries may be exploited for the recovery of such compounds. Inhibitory effects of naturally occurring flavonoids on *in vitro* advanced glycation end-product formation have been studied[53].

Several mechanisms involved in the genesis of oxidative stress, such as glucose antioxidation, protein glycation and formation of advanced glycation end-products may be taking place in the diabetic patients. Protein glycation and oxidation could be inhibited effectively when flavonoids were added during the glycalation process, probably due to their antioxidant properties[53]. The role of flavonoids, biflavonoids, flavonolignans, coumarins, bicoumarins, etc. including the biochemistry of their reactions in the metabolic pathways may be studied in detail.

R&D in Medicinal Chemistry of Biflavones and Bicoumarins – Process Development Approach

The leaf extract of *Ginkgo biloba* under the name 'Tebonin' in Germany is used as a cure for blood flow irregularities[56]. This extract was also effective against cerebral blood flow deficiency and its long-term treatment could cure chronic vasotropic ulcers. The extract has also been used in USA for neurological disorders[57]. Biflavones[49-54, 58-61], major constituents of this extract, showed blood flow regulating properties. Since flavones are known to show some antagonism towards certain peptides, the biflavones were also tested for such properties against bradikinin, which is active in capillary permeability and blood flow circulation. Of the biflavones tested, cupressuflavone was found to be the most active bradikinin antagonist[59]. The use of this extract in USA for cerebral insufficiency and against Alzheimers disease has been reported[60, 64-66]. Ginkgetin was the first biflavone isolated from the leaves of *Ginkgo biloba*[56]. Several biflavones have also been isolated[55,60-66].

6, 6"-Binaringenin was isolated from the seed kernels of *Rhus succedanea* (Anacardiaceae)[63]. The synthesis of biapigenin and binaringenin hexa methyl ether were carried by the author and his coworkers using $[Fe(DMF)_3\ Cl_2]$ $[FeCl_4]$ and manganese tris acetyl acetonate (MTA) as the coupling agents[67]. These are the two effective coupling agents that afford good yields of products in less time. Biflavones from medicinal plants have been found to be inhibitors of non-enzymatic lipid peroxidation[68].

Considering importance of the pharmacological properties of biflavonoids[49-54,57,64-68], it is important to make available these compounds in larger quantities. If the compound has been found to possess medicinal properties and is not toxic and has been finally cleared after clinical trials and other tests, the manufacturing process of this compound may be designed for the industry. Process conditions may be optimized. Reactor design including scale up studies may also include kinetics studies, mass transfer, separation engineering, heat transfer, optimization, fluid mechanics and detailed reaction engineering

studies. Process development engineering may be based on shortening the routes of synthesis and also improving the yields of the products by improving the selectivity and inhibiting the byproduct formation to cut down the cost of separation of the product from the byproducts.

A continuous process may be designed for faster reactions whereas a semicontinuous or batch process may be designed for slower organic chemical reactions. The choice of packed bed or fluidized bed reactor would again be based on the different catalytic or non-catalytic reactions involved in the synthesis or manufacturing of a pure compound such as a flavonoid, flavonolignan, bilflavone, bicoumarin etc. Synthesis may also be dictated by the environmental considerations in using only (green) non-polluting chemicals. Extractors for the extraction from the plant materials may be based on the semi continuous flow-through reactors (extractors), percolation reactors, columns, trickle bed reactors etc. Effect of particle size of the plant material (biomass) on the extraction of natural product at different time periods, its modeling may be studied for the process development engineering. Fluidized bed extractors may also be designed, if required.

Studies on the oxidative coupling reactions of simple phenolic compounds by using the effective coupling agents (and catalysts) may help in developing the simpler, convenient and cost effective routes and processes for the manufacture of complex compounds such as biflavonoids, bicoumarins, flavonolignans etc. which may be interesting for the studies of the pharmacological activities.

Some compounds in vegetable drugs contain coumarin units[63, 69-72]. Dicoumarol and its derivatives were used clinically to prevent the incidents of thrombosis and to aid dissolution of clots already formed (anticoagulant activities). Hence, considerable interest was evoked in the discovery of bicoumarins for their possible physiological action[63, 69-72].

Seeds[72] of *Euphorbia lathyris* yield 5,5'-bicoumarin, euphorbetin and 5,8'-bicoumarin, isoeuphoretin. Synthesis[68] of 5,5'-linked bicoumarin using $[Fe(DMF)_3\ Cl_2]\ [FeCl_4]$, MTA and $K_3Fe(CN)_6/Na_2CO_3$ as the coupling agents resulted in synthesis of dimer of esculetin, euphorbetin, along with chloroesculetin and a trimer of esculetin-methyl-ethers[73]. Process development engineering work may demand the development of convenient synthesis routes, which may be economical and easy to be scaled up. Reaction engineering studies may require kinetic studies of reactions and optimization studies as well. Biomimetic synthesis of some novel coumarin dimers (bicoumarins) has been reported[74]. Citrus fruit and other plants also contain bicoumarins[75]. Coumarins and bicoumarins from *Ferula sumbul* were found to show anti-HIV activity and inhibition of cytokine release[76].

Oxidative coupling has been invoked as the most plausible path of biosynthesis of various classes of natural products such as flavonoids, coumarins, etc. Even the oxidative coupling reactions are involved between flavones and lignans for the synthesis of flavonolignans. The use of coupling agents such as $[Fe(DMF)_3\ Cl_2]\ [FeCl_4]$ in the oxidative coupling of β-resacetophenone, β-resacetophenone-4-methyl ether, umbelliferone and 4-methyl umbelliferone, β-naphthol, vanillin and feulic acid was made[77]. Vanillin, β-naphthol and ferulic acid showed the coupling reaction. The use of MTA was also made for the coupling reactions. $[Fe(DMF)_3\ Cl_2]\ [FeCl_4]$ was found to be a better coupling agent than MTA. The use of coupling agents may be made to synthesize and manufacture dimeric compounds such as bichalcones, biflavones, bicoumarins, biflavonolignans, etc. Role of biotic and abiotic catalysts in transformation and synthesis of phenolic compounds through oxidative coupling reactions have been studied.[78] Oxidative coupling reactions have also been studied in the biomimetic approach to configurationally unstable or axially chiral bioactive compounds[79]. Novel oxidative coupling agent such as phosphomolybdic acid on silica support has been reported[80]. Oxidative coupling reactions have their own importance in organic and biosynthesis[78,81,82]. Research work on photo-oxidation of chalcone epoxides was also reported[83]. The flavonolignans may be synthesized by the oxidative coupling and other reactions between flavonoids and lignans by designing various synthesis reactions.

Euphorbia lathyris has been reported to be a potential candidate for petrofarming[3-6] and also contains bicoumarins[72]. Thus, there exists a scope for studying petrocrops for investigating the presence of natural products such as terpenes, chalcones, coumarins, flavonoids, alkaloids, etc. which may have pharmaceutical properties including antiparasitic, antiviral and antibacterial properties. Flavonolignans from the seeds of *Hydnocarpus wightiana* were found

to show the pharmacological properties and these seeds also contained hydnocarpus oil, which may be studied as biolubricant and biodiesel. *Spirulina platensis* and other algal species seem to be promising candidates for the production of hydrocarbons, amino acids, proteins, β-carotene, vitamin B-12 and hydrogen[84-86]. Thus, plants may be studied for not only biopharmaceuticals and drugs but also for biofuels.

Vitamin P Activity of Flavonoids

In fact, flavonoids have beneficial health effects. Vitamin P activity of flavonoids was demonstrated in 1950-51 by using some citrus flavonoids, which gave considerable protection against the toxic effects of luekotaxine, bacterial polysaccharide and ionizing radiation[87]. Vitamin P activity was most evident when chemical lesions in the capillary wall were present and increased capillary fragility existed.[87] Biflavonoids have also been associated with Vitamin P activity which may be beneficial in case of high blood pressure and inflammation. Flavonoids have been shown[88] to be more potent anti-oxidant, anti-inflammatory and having antiviral activity. However, more studies are needed to investigate the absorption and metabolism of various flavonoids in individual foods and combination of foods offering different flavonoids to humans in combination.

Flavonoids not only help in promoting healthy circulation, and immune function but also appear to regulate gene expression (Internet – Research Papers of polyphenol and OPC). Rohdewald[89] reported that pycnogenol significantly reduced edema in elderly women and also eased the feeling of geraviness in subjects (> 77.7%). There is lot of information in literature and on the Internet on the beneficial health effects of flavonoids, however, there is a need to extend more studies in this direction, especially on the vitamin P activity of flavonoids as an aid against high blood pressure and inflammation.

Biotechnology of Flavonoids

The role of favonoids such as flavonols, isoflavones, biflavones, their glycosides, bicoumarins, furanocoumarins, lignans and flavolignans in the prevention or treatment of different diseases has been highlighted from time to time[24,28,38-40]. The biological properties of coumarins such as linear furanocoumarins in sensitizing the cells of the human skin to UV radiation and inducing repigmentation of discoloured skin have been reported[90]. Some of the natural products are known for their fungicidal, mullusicidal, antiprotozoal and insecticidal properties[24,25,90]. Furanocoumarins seem to be interesting compounds for studies in the treatment of diseases such as vitiligo, psoriasis, mycosis, fungoides, atopic eczema, lichen planus, pityriasis lichenoides, parapsoisis en plaques, urticaria pigmentosa, alopasia areata, cardiology and neurology (e.g. sclerosis multiplex) etc[90]. Furanocourmarin epoxides also possess antiprotozoal activities[24,25].

The lignans and podophyllotoxins have been reported[90] to possess cytotoxic activity which is of interest for developing anticancer drugs[24,25,90]. Lignans also have antiparasitic activity[24-26]. The oxidative coupling of flavonoids and lignans during biogenesis to form flavonolignans may lead to biosynergistic biological actions.

The polyvalent action of *Ginkgo biloba* flavonoids (ginkgo flavonol glycosides, biflavones etc.) and terpenes and neem (*Azadirachta indica*) has been known[7,50,56,57,90]. *G. Biloba* leaf and root extracts have been of great interest for therapeutics because of their radical scavenging (antioxidant activity), improved blood flow, vasoprotection and PAF activity[50,56,57,90]. Biochemical actions of individual flavonoids as well as their mixtures may be studied in detail as these are normally consumed as mixtures in fruits, vegetables, etc. Bioenergetics of their actions may also be studied.

Wine contains polyphenolic compounds including flavonoids[11]. The moderate consumption of wine under medical advice may provide such grapevine flavonoids, which may lead to protection against cardiovascular diseases and cancer as suggested by epidemiological studies[11,90]. However, further studies in this direction are required to establish these beneficial effects. Biochemical studies on flavone derivatives may also be made which may include combinatorial chemistry approach as well.

Medicinal plants of different categories such as trees, herbs, shrubs, climbers, etc. have been used as traditional medicines[1,7]. Different plant parts such as bark, fruits, stems, leaves, flowers, rhizomes, seeds, wood and even whole plants have been used as medicines[1,7,90]. There is a need to find out the active principles from the plant extracts and carry out

biotechnological studies such as chromatographic separation of extracted compounds as pure compounds (including stereoisomers), their identification, pharmacological studies to find out active principles, toxic effects and antimicrobial properties, structure-activity relationship, biochemistry, organic synthesis, reactor design and scale up of manufacturing process for drug production in pharmaceutical industries.

Bioengineering of medicinal plant products may lead to the development of newer drugs to fight the challenging diseases such as cancer, neurological disorders, heart diseases, HIV-AIDS etc. However, the role of enzymology, cofactors more importantly the flavonoid-protein interactions, bioenergetics, tissue culture, biochemical metabolic pathways (and their mechanisms), chemical versus biosynthesis, chiral engineering, genetic engineering, phytochemistry, microbiology, phramacology etc. may have to be studied in detail.

The beneficial effects of genistein, an isoflavone in soya, cabbage, cauliflower and broccoli have been realized lately. Researchers at the Georgetown University, Washington, DC, USA have reported the ability of these compounds such as genistein and indole-3-carbinol present in these foods to repair the damaged DNA and which may prevent cells turning cacerous[91]. *Ruta graveolens* had been used as traditional medicine for centuries for almost every ailment known to the man. Later on the toxic properties of plant were found. The plant contains rutin and has been used in homeopathy. The biotechnological studies on the root extracts have been reported[92,93]. *R. graveolens* root extracts contain furanocoumarins and have been studied for their activity against intracranial tumours and neurocysticercosis[91,92]. The roots of *R. corsica* contain 5 furnaocoumarins, 3 bicoumarins, 3 quinoline alkaloids and 1 sinapyl sucrose as plant secondary metabolites[94].

Chemistry and biological activity of natural and synthetic phenyloxy coumarins and prenyloxy coumarins have been reported recently[95]. The study of structure – activity relationships found these coumarins to have anti-tumour, anti-inflammatory and antiviral activity. Furanocoumarins have been found to possess good biological activity and thus, these seem to be promising biomolecules for future biotechnological studies to develop medicines. Studies on the photochemistry and photobiotechnology of furanocoumarins may be extended especially against skin diseases. Studies on the tissue culture of the plant cells of *Ruta graveolens* to enhance the production of these plant secondary metabolites have been reported[96]. Ekiert *et al*[96] have reported the accumulation of biologically active furnaocoumarins in *R. graveolens* ssp. Divaricata (Tenore) Gams *in vitro* culture recently. Studies may also be extended on biodimers such as biflavones and bicoumarins.

The flavonoids such as kaemferol, quercetin, their mono-, di- and triglycerides, apigenin and luteolin, besides biflavones such as ginkgetin, bilobetin, isoginkgetin and sciadopitysin have been isolated from the leaves of *Ginkgo biloba*. These are also among the active principles present in *G. biloba* leave extracts. Leave extracts have beneficial effects on subject with senile cognitive decline[90]. Studies have been carried out on the biotechnology of flavonoids, e.g. gene transfer studies on *G. biloba*[90] to enhance the production of secondary metabolites having pharmacological activity. However, further research in the area of tissue culture and genetic engineering of medicinal plants needs to be extended for enhancing the production of flavonoids for industrial production. Studies on plant physiology, genetic engineering, tissue culture, photobiology (photosynthesis), metabolic engineering etc. may be extended for enhancing the yields of desired bioflavonoids in the plants.

Bioinformatics studies may help in cutting down the time, efforts and cost of finding out new drugs where the natural products such as flavonoids and coumarins may be studied. Trypnocidal activity of 22 extracts and 43 fractions of plants belonging to the families *Meliaceae* and *Rutaceae* have been reported recently[97]. Two flavonoids were isolated from these extracts. It was revealed that order Rutales is a promising source of new drugs for Chagas disease. Structure-activity relationships of novel inhibitors of glyceraldehyde -3- phosphate dehydrogenase (GADPH) have been studied using 3D QSAR studies, which were performed on a library of 120 GADPH inhibitors, including a series of coumarins such as chalepin (furanocoumarin derivative), flavonoids and their derivatives[98]. Principal component analysis (PCA)/Partial least sqaure (PLS) regression analysis was used for the evaluation of the structure features

crucial for potency, selectivity and favorable pharmacokinetic properties. These are important for the design of new ligands and drugs through bioinformatics studies. Docking studies using furanocoumarins such as 3-piperonyl coumarins as inhibitors of glycosomal glyceraldehyde-3-phosphate dehydrogenase from *Trypnosoma cruzi* have shown good results[99,100]. Some flavonoids have also been used for docking studies to find out the suitable inhibitors. These studies may lead to drug designing for Chagas disease. The interesting inhibitor compounds found out by bioinformatics studies may be studied experimentally in the laboratory for drug development.

Conclusions

Natural products including flavonoids offer a wide scope for the drug research. There is a scope of integrating drug development with petrofarming and biodiesel production. *Euphorbia lathyris*, which provides not only biocrude for production of petroleum hydrocarbons but also bicourmarins, may have pharmacological properties. Convenient routes of synthesis of natural products may pave the way for chemical reaction engineering, modeling and process (design) development studies.

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