Chapter – 2

REVIEW OF LITERATURE

2.1 *Bischofia javanica* Blume:

Synonym: ***Bischofia trifoliata***
Family: Euphorbiaceae
Common Name: Bishop Wood
Nepali: Kainjal; **Hindi**: Bhillas

Distribution:

South-west Asia, Japan, Australia. In India it is distributed over the Sub-Himalayan region, Orissa and south-west coast from Konkan to Nilgiris. [1] The photograph of the leaflets of *Bischofia javanica* is presented in **Figure 2.1.**

Botanical Description:

Evergreen tree commonly 12-18 m in height, with dense, round headed, smooth branches and milky sap. Leaves are alternate, long petiole, and trifoliate, leaflets are shiny, oval elliptic, 15-20cm long, with margins small toothed. Flowers tiny, without petals, greenish yellow in color. Fruits pea sized, fleshy about 9 mm in diameter. [2]

Phytoconstitutents:

Tannin, β amyrins, betulinic acid, chrysoeriol, ellagic acid, fiestin, friedelan-3α-ol, epifriedelinol, friedelin, luteolin and glucoside, quercetin, quercitrin, beta-sitosterol, stigmosterol, ursolicacid, triacontane, [3-7] fatty acids including linoleic acid, stearic acid, [8] bischofianin, corigalin, furosin, geranin and punicalagin [9] are the reported isolated phytoconstituents.

Traditional Uses:

The bark is used for the treatment of stomach ulcer, mouth ulcer and athlete's foot. It is also used in the treatment of tuberculosis. [10-11] Leaves are used in the treatment of stomach ache; leaf juice is used in the treatment of cancerous wounds. [12] Bark of this plant is used as abortificient and emmenagogue. [13] Bark is also used in fever, cough, throat ailments, tooth ache, burns, sores, eye diseases and diarrhea. [14]

Pharmacological Review:

Antimicrobial activity of ethanolic extract of leaves of *Bischofia javanica* was performed, by Khan *et al.* [15] The extract shown to have action against both gram positive and gram negative microorganisms.

DNA Topoisomerase II is target enzyme for anticancer chemotherapeutic drug development. Bioassay-guided fractionation of the chloroform extract of the bark of *Bischofia javanica* led to the isolation of Betulinic acid and its derivatives, betulonic acid, 3β-O-(Z)-coumaroylbetulinic acid and 3β-O-(E)-coumaroyl betulinic acid. These compounds were found to be catalytic inhibitors of Topoisomerase II activities with IC_{50} values ranging from 0.38 to 58μM. The acylation of the OH group at C(3) of betulinic acid exhibited stronger Topoisomerase II inhibitory activity. [16]

Figure 2.1 Photograph showing the leaflet of *Bischofia javanica* collected from the Melli region of Sikkim

2.2 *Fraxinus floribunda* Wallich:

Synonym: ***Fraxinus bungeana***

Family: Oleaceae

Common Name: Himalayan Ash

Nepali: Lakuri

Distribution:

The genus Fraxinus (Oleaceae) is distributed mostly in the temperate regions and the subtropics of the Northern hemisphere. *Fraxinus floribunda* is distributed in East Asia-Himalayas from Himachal Pradesh to South-west China. In Sikkim sub alpine region at altitude of 4000-8000 ft. [17] The photograph of the leaflets of *Fraxinus floribunda* is presented in **Figure 2.2.**

Botanical Description:

A deciduous tree growing up to 40 m height. Leaves are pinnate, leaflets are 7-9, opposite, stalled, ovate-oblong. [18] Inflorescences are terminal or axillary toward end of branches, or lateral on branches of previous year, paniculate, bracts linear to lanceolate, caduceus or absent. Flowers are small, unisexual, bisexual, or polygamous. The calyx is 4-toothed or irregularly lobed, sometimes absent. The corolla is white to yellowish, 4-lobed, divided to base or absent. Stamens are two, inserted at base of corolla lobes; filaments are short. Ovules are two in each locule, pendulous. The fruit is a samara with elongated wing. The seed is usually one, ovate-oblong, endosperm fleshy, radicle erect. [19]

Phytoconstituents:

The presence of coumarins, secoiridoids, and phenyl ethanoids is a characteristic feature of Fraxinus species. Coumarins, which have been isolated from *Fraxinus floribunda* are esculetin, esculin, fraxetin, fraxin, 8-Acetyl-7-hydroxy-6-methoxycoumarins, 8-methoxycoumarins, floribin. [20] The triterpenes

ursolic acid, oleanolic acid, betulinic acid , betulin were also reported in some species of Fraxinus. [21]

Traditional Uses:

Many species of Fraxinus attract considerable attention for their medicinal properties and find application in the folk medicine, as well as in the contemporary medicine [22-23]. The Fraxinus species have been used in folk medicine in different parts of the world for their diuretic and mild purgative effects as well as for treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp. Manna obtained by incision from the stem of the tree is used as laxative. Barks and leaves of the plant are traditionally used for the treatment of fracture and dislocation, leaves are employed as diuretic and for the treatment of gout. [24]

Pharmacological Review:

The Fraxinus species (*F.jabonica, F. excelsior, F. ornus*) shown to have potent anti-inflammatory activity in animal models, moreover the crude extracts inhibited the lipoxygenase enzyme involved in the arachidonic acid pathway was found in that species.[25] The liver protecting properties of some coumarin and flavonoid components of Fraxinus spp. are reported. The methanol extract of the bark of *F. japonica* was bio assayed for inhibitory activity on rabbit platelet aggregation induced by arachidonic acid and found to be active. Traditionally, leaf extracts from *F. excelsior* have been used to facilitate renal excretion. [26]

A high inhibitory activity against cAMP-phosphodiesterase was shown by the coumarin compounds of the bark of *F. japonica* scopoletin, fraxin and isofraxidin. Investigations of Meyer *et al.* reveal the antioxidative activities of the alcoholic extract of *F. excelsior* bark, a component of the anti-inflammatory plant drug Phytodolor N. [27] The ethyl ether fraction of the alcoholic extract of *F. excelsior* bark was inhibitory to *Bacillus subtilis*. Studies carried out by Lambrev *et al.* revealed a clear antibacterial activity of the ethanol extract and decoctions from the bark of *F.*

ornus against *S. aureus* and *B. subtilis*, as well as a marked activity against *Leptospira ponoma.* [28]

The pharmacological review revealed that no research work was performed on anti-inflammatory and anticancer activities of *F.floribunda.* Moreover, all the phytochemicals of the plant are also not fully explored. Therefore *F.floribunda*, the plant which is extensively used in ethno-medicine by the tribes of Sikkim has been undertaken in this study to explore its phytoconstituents and their pharmacological activities.

Figure 2.2 Photograph showing the leaflet of *Fraxinus floribunda* in branches collected from Pakyong region of Sikkim.

2.3 Updated Perusal on Anti-inflammatory Studies:

Inflammation occurs with three phases of different vascular and cellular characteristics. The acute phase is characterized by vasodilation and increased vascular permeability, the sub-acute phase is characterized by the infiltration of leukocytes and phagocytic cells and a chronic proliferative phase in which tissue degeneration and fibrosis occurs. Models for studies of anti-inflammatory activity of a drug can be classified in to three types. [29]

1. Acute model: UV-Erythema in guinea pig, croton oil induced edema in rats or mice paw edema in hind limbs of rat induced by carrageenan, histamine, serotonin, egg white, and formalin etc, pluristy tests in rat.

In acute model the most common method employed to screen anti-inflammatory activity is Carrageenan induced paw edema, here the reduction in paw volume after drug treatment is studied by using Plethysmograph.

2. Sub-acute model: Cotton pellet induced granuloma in rats, glass rod induced granuloma in rats and PVC sponge induced granuloma in rats.

In this model cotton pellet induced granuloma is the most commonly employing method. Implantation of sterile cotton pellets is done subcutaneously under the skin in either side of groin region of rats. After seven days of drug administration cotton pellets with granuloma is dissected out, dried and weighed. The protection against granuloma by the drugs is thus determined from difference of weight of the cotton pellets.

3. Chronic models: Adjuvant induced arthritis in rats, experimental allergic encephalitis and delayed type hypersensitivity.

Freund's adjuvant (Freund's adjuvant is a water-in-oil emulsion consisting of a mineral oil, an antibody stimulator such as tubercule bacilli, and an emulsifying agent such as lanolin or Arlacel-A) induced arthritis in rats is the common method in

chronic model of anti-inflammatory screening of drugs. Here after 21 days of drug administration decrease in paw volume is studied by a plethysmograph.

The drugs with anti-inflammatory activities may have anti-nociceptive and antipyretic activities. This can be supported by the mediators involved in inflammation like PGs, vasoactive amines, leukotrienes, interleukins and TNFα which involved in pain and pyrexia.

The probable mechanism of most of the anti-inflammatory agents of natural origin are interfering the arachidonic acid pathway of PGs and other mediators synthesis. It may inhibit non selectively COX-1, COX-2, 5-LOX, which can be studied by both *in vivo* and *in vitro* models. [30-32]

The literatures on the anti-inflammatory activity of some plant drugs are reviewed here. Besra et al have screened the petroleum ether extract of leaves of *Lichi chinensis* for anti-inflammatory property in acute and sub acute model in rats. The study revealed that the extract of *Lichi chinenesis* had significant anti-inflammatory activity in the dose range of 50,100 and 200mg/kg i.p. [33] Moreover the extract shown to have potent analgesic activity in acetic acid induced writhing .The COX pathway responsible for the anti inflammatory activity was also established in this study.

A group of medicinal plants from Samoan and Peruvian region were screened for inhibition of prostaglandin biosynthesis and rat ear edema by Christina AD and his co-workers. The study revealed that 14 plants showed moderate to strong inhibition of COX-1 enzyme in the *in-vitro* assay. Further, 10 Samoan and 8 Peruvian plants have inhibited the ethyl phenyl propionate induced ear edema *in vivo*. [34]

The anti-inflammatory activity of an alcohol extract of *Achyranthes aspera* was tested on carrageenin induced hind paw oedema and cotton pellet granuloma models in albino rats. The paw volume was measured plethysmographically at 0, 1, 2, 3, 4 and 5h. In the sub- acute model cotton pellet granuloma was produced by implantation of

50±1mg sterile cotton in the axilla under ether anesthesia. The animals were fed with an alcohol extract at various dose levels (125,250,375 and 500 mg/kg).Dicolfenac sodium was used as standard drug. The alcohol extract (375 and 500mg/kg) showed the maximum inhibition of oedema by 65.38% and 72.37% at the end of 3h respectively. Using a chronic test, the extract exhibited a 40.03% and 45.32% reduction in granuloma weight [35] at the dose of 375 and 500mg/kg respectively.

The chloroform, methanol and ether extracts of *Venonia cinerea* leaf at the doses of 100,200and 400mg/kg i.p were tested for acetic acid induced writhing in mice, carrageenan induced oedema and brewers yeast induced pyrexia in rats. The results revealed that the extracts have potent analgesic, anti-inflammatory and antipyretic activities compared to control group. [36]

Choi J and his co-workers have isolated Nigaichigoside (1,23-hyrdroxytormentic acid 28-o-glc) and 23-hydroxytormentic acid from the fruits of *Rubus coreanus*. These compounds shown to have potent anti-inflammatory activity in Carrageenan induced paw edema and antinociceptive effects in hot plate and tail-flick models. Moreover the isolates shown to have reducing lipid peroxidation and hydroxyl radical formation. [37]

Anti-nociceptive and anti-pyretic evaluations were performed on the aqueous and alcoholic extracts of *Emblica* officinalis.[38] A single oral dose of ethanol and aqueous extract at the dose of 500mg/kg i.p showed significant reduction in brewer's yeast induced hyperthermia in rats. But the extracts did not show any significant analgesic activity in the tail immersion test. In writhing method the extract shown significant analgesic activity.

Anti-nociceptive, antipyretic and anti-inflammatory activities were screened for the different extracts of leaves *Aegle marmelos* at the dose of 50mg/kg i.p against the standard drugs paracetamol of anti-nociceptive and antipyretic studies and Phenylbutazone for anti-inflammatory studies. [39] The extracts produced marked analgesic activity by reducing the early and late phases of paw licking in mice. A

significant reduction in hyperpyrexia in rats was also produced by the most of the extracts. Carrageenan induced paw edema and cotton pellet induced granuloma were also reduced by the extracts of leaves of *Aegle marmelos.*

Various *in vitro* and *in vivo* anti-inflammatory studies were performed on the aqueous extract of pericarps of *Sapindus trifoliatus. In vitro* inhibitory activity was studied against 5-Lipoxygenase (5LO), Cyclo-oxygenase (COX), Leukotrienes B4 (LTB4) and nitric oxide synthase (NOS) [40]. At doses of 20 and 100mg/kg i.p *S. trifoliatus* was evaluated for the acute pedal inflammation induced by carrageenan, histamine, serotonin and zymosan in rats and mice. Both the *in vitro* and *in vivo* studies revealed the potent anti-inflammatory activity. Moreover, 5-LOX and COX inhibiton was found to be the probable mode of action.

Anti-inflammatory and analgesic activities as well as the median lethal dose (LD_{50}) of water–ethanol extract of the aerial parts of *Pothomorphe umbellata* were evaluated in animal models. [41] The ED_{50} (oral) for the inhibition of carrageenan-induced rat paw edema was determined to be 550 mg/kg, while the LD_{50} was higher than 2.0 g/kg. At a dose of 550 mg/kg, inhibited the inflammatory process by 48.7% on the third hour of the assay (edema peak) when compared to the untreated control. Indomethacin, the positive control used in this test, inhibited the edema by 58.6% at a dose of 10 mg/kg, when compared to the untreated control. All three fractions – hexane, methylene chloride and ethyl acetate showed inhibition of the edema induced by carrageenan over a period of 4 h but the methylene chloride fraction showed the best activity. The activity shown by the methylene chloride fraction at 200 mg/kg was comparable to that exhibited by indomethacin at a dose of 10 mg/kg. The number of writhings induced by a 0.6% acetic acid solution on intraperitoneal injection was decreased by 22% in the group treated orally with *Pothomorphe umbellata* crude extract. It also inhibited the granulomatous tissue formation in rats by 6.2%. In the same assay, topically applied dexamethasone decreased the granuloma formation by 14.2%.

Anti-inflammatory activity of both glycerrhizinic acid and the aqueous glycerrhiza extract was compared with diclofenac sodium (10 mg/kg), using the carrageenan-induced paw edema model in male albino rats. The result of the study depicted that glycerrhiza had significant anti-inflammatory activity. [42]

Anti-inflammatory and antitumor activities of seven triterpene acids from the leaves of *Eriobotrrya jabonica* was studied by Banno N and his team. [43] All the tested compounds showed a marked anti-inflammatory effect, with a 50% inhibitory dose (ID_{50}) of 0.03-0.43 mg /ear.

The root of *Morinda officinalis* (Rubiaceae) is used to treat rheumatoid arthritis and impotence in the traditional oriental medicine. To identify the antinociceptive and anti-inflammatory components of the crude drug, activity directed fractionation was done .The active fraction of the Butanol extract of *M.officinalis* root was subjected to silica gel and ODS column chromatography to yield two compounds and were identified as monotropein and deacetylasperulosidic acid, respectively. The iridoid glycoside, monotropein was tested for its anti-inflammatory and antinociceptive effects using hot plate and writhing assays and by using carrageenan induced anti-inflammatory assays in mice and rats. The study revealed that monotropein at doses of 20,30 mg/kg p.o significantly reduced the pain and inflammation. [44]

Acute and chronic inflammation models were used to evaluate the anti-inflammatory activity of the methanol extract of *Plumeria acuminate* by Gupta M and her co-workers. [45] In acute model carrageenan, dextran, histamine and serotonin were used to induce inflammation in rat hind paw and cotton pellet-induced granuloma method was used for chronic inflammation model. The methanol extract of *Plumeria acuminata* exhibited significant anti-inflammatory activity on the experimental animal models. The extract (500 mg/kg b.w) exhibited maximum anti-inflammatory effect i.e., 30.51, 47.06, 34.48 and 32.50% at the end of 3 h with carrageenan, dextran, histamine and serotonin respectively. Administration of MEPA (500 mg/kg b.w) and indomethacin (10 mg/kg b.w) significantly reduced the formation of

granuloma tissue induced by cotton pellet method at a rate of 45.06 and 51.57% respectively. The effect produced by the extract was comparable to that of indomethacin a prototype non-steroidal anti-inflammatory agent.

Different fractions obtained from the leaves of *Kalanchoea crenata* was screened for anti-inflammatory activity in carrageenan induce paw edema in rats. n-butanol fraction shown to have potent anti-inflammatory activity compared to other fractions. [46] The n-butanol fraction (600mg/kg) was further screened for the activity in inflammation induced by histamine, serotonin and formalin. The protection against histamine, serotonin and formalin induced inflammations are 47.51%, 54.71% and 40.00% respectively.

Tabernaemontana coronaria extracts were studied for anti-inflammatory and anti-oxidant activities. In this study Paw edema thickness was measured using vernier callipers in carrageenan and formalin induced paw edema in rats. The study revealed the reduction in paw thickness after drug administration of 1,2 and 3hrs.The alcoholic extract of the drug inhibits paw edema by 71% at the dose of 250mg/kg p.o [47]

One of the major factors limiting the use of non-steroidal anti-inflammatory drugs is gastrointestinaltoxicity.Gaultherin,2-[(6-O-β-DXylopyranosyl-β-D-glucopyranosyl) oxy]benzoic acid methyl ester, a natural salicylate derivative extracted from *Gaultheria yunnanensis*, has been shown to have analgesic and anti-inflammatory effects and lack gastric ulcerogenic effect compared to aspirin. The mechanism of action of gaultherin has been studied and the results showed that gaultherin (200 mg/kg) significantly inhibited the abdominal contractions in the acetic acid induced writhing test in mice. The anti-inflammatory effect of gaultherin was demonstrated in the croton oil-induced ear edema model in mice. The results showed that gaultherin and equimolar dose of aspirin produced comparable inhibitory effects. The study of the metabolism characters of gaultherin in mice and rats indicated that gaultherin could be metabolically converted to salicylate, which produced the pharmacological effects, and provided effective concentrations for an extended period. *In vitro* metabolism experiment showed that gaultherin was metabolized by β-glycosidase

produced by human intestinal bacteria and esterases in intestine, blood and liver successively to release salicylate finally. The study suggested gaultherin did not cause gastric ulcer for the reason that it released salicylate in intestine slowly, not in stomach and it left the cyclooxygenase-1 unaffected, which was the source of cytoprotective prostaglandins in gastric epithelium. [48]

The anti-inflammatory effect of extract and fractions of the root bark of *Securidaca longipedunculata* Fres (Polygalaceae) was performed by Okali *et al.* The extract and fractions inhibited topical edema induced by xylene in the mouse ear. [49]

Anti-inflammatory activity of gardenia fruit and the phytoconstituents genipin, and geniposide were studied. Both genipin and geniposide inhibited production of exudate and nitric oxide (NO) in the rat air pouch edema model. However, genipin possessed stronger anti-inflammatory activity than geniposide, as demonstrated by the results with carrageenan-induced rat paw edema, carrageenan-induced air pouch formation, and measurement of NO content in the exudates. [50]

Lupane triterpenoids were isolated from *Maytenus* species.[51] The isolated triterpenoids significantly inhibited the inflammatory mediators NO, PGE_2 and proved the ethno-medicinal utility of the plant species for different types of inflammatory disorders.

Anti-inflammatory activity of alkanoids and triterpenoids from *Trichodesma amplexicaule* Roth was performed.[52] The result of the study revealed that triterpenoids at the dose of 5, 10, 15mg/kg p.o significantly reduced the carrageenan and Freund's adjuvant induced inflammation in rats.

The anti-inflammatory activity of the aqueous extract of *Polygala japonica* in mice and rats was studied to find the pharmacological basis for its ethno medical use. The extract produced a significant inhibition of peritoneal and cutaneous vascular permeability induced by acetic acid and histamine respectively and ear swelling induced by picryl chloride in mice at the dose of 25.0 mg/kg. Moreover, the extract

markedly inhibited the footpad edema induced by histamine in rats, and decreased prostaglandin E2 (PGE2) content in carrageenan-induced air-pouch. [53]

The anti-inflammatory activity of pentacyclic triterpene, oleanolic acid was examined on acute nociception induced by intraplantar injection of capsaicin in mice. The data suggested that oleanolic acid inhibits capsaicin-evoked acute nociception due to mechanisms possibly involving endogenous opioids, nitric oxide, and K_{ATP}-channel opening. [54]

The anti-inflammatory activity of hexane leaf extract of *Aspilia africana* was evaluated in rodents using the xylene induced ear edema egg albumin- and agar-induced paw edema, formaldehyde-induced arthritis, cotton pellet granuloma, gastric ulcerogenic, acetic acid-induced vascular permeability and dextran-induced *in vivo* leukocyte migration tests. [55] Results showed that the extract (5 mg/ear) inhibited topical edema in the mouse ear and at 200 and 400 mg/kg (i.p.), it significantly suppressed the development of egg albumin-and agar induced paw edema, and the global edematous response to arthritis induced by formaldehyde in rats.

The ethanol extract of *Thespesia populnea* bark was investigated for anti-inflammatory and analgesic activity at the doses (p.o.) of 100, 200 and 400 mg/kg body weight. For evaluation of inflammation carrageenan, histamine and serotonin induced paw edema served as acute models and formaldehyde-induced arthritis served as a chronic model in rats. The acetic acid-induced writhing response and formalin-induced paw licking time in the early and late phases of mice were used to assess analgesic activity. The higher doses were inhibiting carrageenan, histamine and serotonin-induced paw edema as well as formaldehyde-induced arthritis successfully. [56]

Anti-nociceptive activity of two Amazonian Copaiba oils *Copaifera multijuga* Hayne and *Copaifera reticulata* Ducke, were studied for peripheral (acetic acid-induced abdominal writhing and formalin), spinal (tail flick) and supra-spinal (hot plate)

models of nociception. Results demonstrated that the Copaiba oils did not develop toxic effects. Doses ranging from 30 to 150 mg/kg were enough to significantly develop peripheral antinociceptive effect. All Copaiba oils demonstrate central activity but with less effect on supra-spinal regions of the brain. Administration of the opioid receptor antagonist, naloxone completely inhibited the antinociceptive effect induced by both Copaiba oils. The results indicated that Copaiba oils demonstrate peripheral and central antinociceptive effect.[57]

The popular medicine *Passiflora edulis* has been used as a sedative, tranquilizer, against cutaneous inflammatory diseases and intermittent fever. Most of the pharmacological investigations of *Passiflora edulis* have been addressed to its Central Nervous System activities, such as anxiolytic, anticonvulsant and sedative actions. The anti-inflammatory effect of aqueous lyophilized extract obtained from leaves of *Passiflora edulis* was studied in the mouse model of pleurisy induced by carrageenan, bradykinin, histamine or substance P. The result revealed that the extract inhibited the leukocyte activation and mediators release during inflammation. [58]

Salvia officinalis L. leaves, obtained from four plant populations of different origin, were investigated for their topical anti-inflammatory properties. The *n*-hexane and the chloroform extracts dose-dependently inhibited the Croton oil-induced ear edema in mice, the chloroform extracts being the most active. In contrast, the methanol extracts showed a very low effect and the essential oil was inactive. Chemical and pharmacological investigation of the most potent chloroform extract, revealed ursolic acid as the main component involved in its anti-inflammatory activity. The anti-inflammatory effect of ursolic acid (ID_{50}=0.14 μMoles/cm^2) was two fold more potent than that of indomethacin (ID_{50}=0.26 μMoles/cm^2), which was used as a reference non-steroidal anti-inflammatory drug.[59]

Anti-inflammatory and anti-nociceptive activities of *Smilax china* L aqueous extract at the dose of 1000mg/kg p.o shown to have significant activities. The inhibition of

PGs production in lipopolysaccharide induced mouse macrophage cells depicted the COX inhibition of aqueous extract of *Smilax china.* [60]

2.4 Updated Perusal on Anticancer Studies:

A normal cell turns into a cancer cell because of mutation takes place in its DNA, which can be inherited or acquired. The characteristics of cancer cells are uncontrolled proliferation, de-differentiation, invasiveness and metastasis.[61] The major mechanisms of action of anti-neoplastic drugs are by induction of apoptosis.

Apoptosis - a Leading Mechanism in Anticancer Drug Discovery:

Apoptosis is a defensive mechanism naturally existing in our body to maintain homeostasis. It is especially important in the context of malignancy because it acts as a first line defense against mutations-removing cells with abnormal DNA that could become malignant. The intracellular pathway of apoptosis involves activation of interleukin-1b enzyme, ceramide, proteases, endonucleases etc. The action of various gene products like the p^{53} protein modulates and controls apoptosis, the Bcl2 gene protein represses apoptosis. There are evidences that, most of the cytotoxic anticancer drugs initiate apoptosis. [62] Apoptotic pathway is one of the thirst areas in new drug discovery for cancer.

Characterization of apoptosis mainly includes morphological and ultrastructural observations. Intracellular changes and plasma membrane structural modifications have been widely recognized as crucial factors involved in cell injury and death. Changes in nuclear morphology and in organelle structure as well as specific phenomena at the cell surface level, namely surface smoothing and surface blebbing, are often considered as markers associated with cell pathology.[63] In addition, it must be recalled that these structural findings are intimately related to the cascade of biochemical and physiological events leading to changes in cellular homeostasis, loss of cell volume regulation, modifications of macromolecule synthesis and, finally, to the loss of cell viability.

A single intracellular event can be extensively analyzed by using, in parallel, biochemical, molecular or ultra structural approaches. The complex sequence of structural modifications ultimately leading to cell death can be recognized by light and electron microscopy techniques. These analysis are mainly qualitative and can indicate: i) the different features of the apoptotic process in terms of appropriate markers e.g. histotype-associated, ii) the staging of the process, e.g. early or late phases (also called secondary necrosis). However, quantitative analysis, i.e. cytometric and morphometric analysis can also be performed by using light microscopy, fluorescence microscopy, confocal microscopy, scanning electron microscopy and, in some conditions, transmission electron microscopy. [64 - 65]

Flow cytometry has been used to detect apoptotic cells either by looking at changes in morphology of the dying cell, that can be detected by alterations in the light scattering properties [66], or by assessing cellular DNA content. The cellular DNA content can be measured following cell fixation that leads to a partial leakage of degraded DNA within apoptotic cells. As a consequence, apoptotic cells contain reduced DNA content and can be recognized, following staining of cellular DNA with intercalating dyes, as the cells with low DNA are highly stained (sub G0-G1 peak). To visualize apoptosis in a subset of an heterogeneous cell population, simultaneous detection of a surface antigen specific for the subset and DNA status should be performed.

Anticancer screening of drugs is performed by multipoint of studies, which confirms apoptosis. Some of the studies are mentioned below which have been supported by the literatures

1. Cell viability study
2. Cytotoxicity study
3. DNA fragmentation assay
4. [3] H Thymidine incorporation assay
5. Fluorescent microscopy
6. Confocal microscopy

7. Western blot study for gene expression
9. Flow cytometry to assess the cell cycle.

2-methoxyestradiol (2-ME) was tested for its anticancer activity on human pancreatic cancer cells. 2-ME inhibited the growth of these cell lines (50–90%) in a dose and time-dependent fashion, and terminal deoxynucleotidyl transferase staining showed that it induced apoptotic cell death. Flow cytometric analysis indicated that 2-ME-sensitive cells showed a prolonged S phase after 48 h of treatment. Using a mouse model for *in vivo* studies on lungs metastasis and injecting MIA PaCa-2 cells into the tail veins of *nu/nu* mice, lungs colonies were formed. Mice given oral dose of 2-ME showed 60% inhibition in the number of lungs colonies compared with control, untreated animals. These results suggest that 2-ME may have clinical application for the treatment of pancreatic cancer. [67]

The Bcl-2 family of proteins regulates a late step in the apoptosis pathway. Bcl-2 protein is believed to be involved in imparting resistance to programmed cell death or apoptosis induced by chemotherapeutic agents and radiation. The anti-apoptotic function of the Bcl-2 protein appears to be modulated by its ability to heterodimerize with other members of the gene family, predominantly Bax, a protein favouring induction of apoptosis. Susceptibility to undergoing apoptosis may, therefore, be dependent on the ratio between Bcl-2 and Bax. Both Bax and Bcl-2 are regulated by the tumour-suppressor protein p53. The study was undertaken to analyse the significance of the Bcl-2:Bax ratio, p53 expression and apoptosis in paediatric acute lymphoblastic leukemia (ALL). Expression of Bax, Bcl-2 and p53 was determined by immunocytochemistry, and apoptosis was evaluated by an enzymatic end-labelling technique using biotin-dUTP and further confirmed by annexin binding. The presence of mutant p53 was determined using a mutant-p53-specific enzyme-linked immunosorbent assay (ELISA). A total of 32 cases and 20 controls were evaluated. Bcl-2 was found to be expressed in 22/32 of the all cases. Pretreatment (spontaneous) apoptosis was observed in 23/32 cases. The mean pretreatment apoptotic index was 2.04 – 11.34% with a median value of 7.5%. [68]

Curcumin, the major component of the spice turmeric, is used as a coloring and flavoring additive in many foods and has attracted interest because of its anti-inflammatory and chemopreventive activities. However, this agent also inhibits the generation of reactive oxygen species (ROS) and the c-Jun NH_2-terminal kinase (JNK) pathway, and because many chemotherapeutic drugs generate ROS and activate JNK in the course of inducing apoptosis. Studies in tissue culture revealed that curcumin inhibited camptothecin, mechlorethamine, and doxorubicin induced apoptosis of MCF-7, MDA-MB-231, and BT-474 human breast cancer cells by up to 70%. Inhibition of programmed cell death was time and concentration dependent, but occurred after relatively brief 3-h exposures, or at curcumin concentrations of 1 μM that have been documented in Phase I chemoprevention trials. Under these conditions, curcumin exhibited antioxidant properties and inhibited both JNK activation and mitochondrial release of cytochrome-C in a concentration-dependent manner. Using an *in vivo* model of human breast cancer, dietary supplementation with curcumin was found to significantly inhibit cyclophosphamide-induced tumor regression. Such dietary supplementation was accompanied by a decrease in the activation of apoptosis by cyclophosphamide, as well as decreased JNK activation. These findings support the hypothesis that dietary curcumin can inhibit chemotherapy-induced apoptosis through inhibition of ROS generation and blockade of JNK function, and suggest that additional studies are needed to determine whether breast cancer patients undergoing chemotherapy should avoid curcumin supplementation, and possibly even limit their exposure to curcumin-containing foods.[69]

The effects of 1,8-cineole in two human leukemia cell lines, Molt 4B,HL-60 and stomach cancer KATO III cells was studied by Hiroyuki, M. and his coworkers. Specific induction of apoptosis by 1, 8–Cineole was observed in human leukemia Molt 4B and HL-60 cells but not in human stomach cancer KATO III cells. Morphological changes showing apoptotic bodies were observed in the human leukemia HL-60 cells treated with 1, 8 cineole. The fragmentation of DNA by cineole to oligonucleosomal sized fragments i.e. a characteristic of apoptosis were

concentration and time dependent in Molt 4B and HL 60 cells, but not in KATO III cells.[70]

Quercetin and flavopiridol, both flavonoids that influence oxidative milieu, proliferation, and apoptosis of various cell types, were examined for their effects on acute myelogenous leukemic cells and normal progenitors. Both quercetin and flavopiridol inhibited the growth and viability of various acute myelogenous leukemia (AML) cell lines and AML blasts isolated afresh from patients with AML of various subtypes. The effects on inhibition of proliferation and decreased viability were also significant in normal CD34+ cells isolated from normal marrow donors. In certain AML cases, the effects of flavopiridol appeared to be mediated through activation of caspase 3, offering one possible mechanism for the apoptosis evident after exposure to flavopiridol as measured by annexin V expression. These flavonoid compounds might find use in various therapeutic settings in AML. [71]

The antiproliferative activity of the methanol extract of *Daphne mucronata* (Thymelaeaceae) was evaluated using human myelogenous leukemia K562 cells. The cells responded to plant extract treatments in a dose dependent manner and the IC_{50} of the crude extract (equivalent to 1 g of plant leaves powder per ml) and the purified active component was found to be 42 µl and 1.3 µM, respectively. The antiproliferative activity of the plant was also evaluated using flowcytometry technique. The results indicated that the crude extract and the active purified component are capable of arresting the cells in G_1 phase of the cell progression cycle. [72]

Methylene chloride fraction of *Scutellariae barbatae* was studied for apoptosis related experiments on human U937 leukemia cells by (a) 2,3-bis[2-4-nitro-5-sulphophenyl]2*H*-tetrazolium-5-carboxanilide (XTT) assay for cytotoxicity; (b) terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay for morphological changes; (c) cell cycle analysis; (d) Western blot analysis of poly(ADP-ribose) polymerase (PARP), caspase-8, caspase-9, caspase-3 and Bax, Bcl-

2 and cytochrome-C expressions for apoptosis signaling pathway. The extract inhibited the proliferation of human U937 leukemia cells in a dose-dependent manner (IC_{50}=10 μg/ml). It dose-dependently increased the sub-G_1 DNA contents by cell cycle analysis. DNA fragments indicating induction of apoptosis was observed in extract treated U937 cells by TUNEL assay. Caspase-9 and caspase-3 were activated while caspase-8 was intact by drug. Similarly, it effectively cleaved PARP, increased the ratio of Bax/Bcl-2 and released the cytochrome-C from mitochondria during apoptosis in U937 cells. The methylene chloride fraction of *Scutellariae barbatae* induced apoptosis via the mitochondria-mediated signaling pathway. [73]

L-Ascorbic acid (LAA) was investigated clinically for the treatment of patients with acute myeloid leukemia (AML) based on the observed effects of LAA on AML progenitor cells *in vitro*. However, the mechanism for LAA-induced cytoreduction remains to be elucidated. LAA at concentrations of 0.25–1.0mM induced a dose- and time-dependent inhibition of proliferation in three AML cell lines and also in leukemia cells from peripheral blood specimens obtained from three patients with AML. In contrast, ovarian cancer cell lines were only minimally affected. Flow cytometric analysis showed that LAA at concentrations of 0.25–1.0mM could significantly induce apoptosis in the AML cell lines. LAA induced oxidation of glutathione to oxidized form (GSSG) and subsequent H_2O_2 accumulation in a concentration-dependent manner, in parallel to induction of apoptosis. [74] The direct role of H_2O_2 in the induction of apoptosis in AML cells was clearly demonstrated by the finding that catalase could completely abrogate LAA-induced apoptosis. Induction of apoptosis in LAA-treated AML cells involved a dose-dependent increase of Bax protein, release of cytochrome-C from mitochondria to cytosol, activation of caspase-9 and caspase-3, and cleavage of poly [ADP-ribose] polymerase. In conclusion, LAA can induce apoptosis in AML cells, and this is clearly due to H_2O_2, which accumulates intracellularly as a result of oxidation of reduced glutathione by LAA.

The total alkaloid fractions of the methanol extract of the leaves, ripe fruits, roots, seeds and stem of *Solanum pseudocapsicum* were subjected to *in vitro* cytotoxicity, short term toxicity and long term survival studies. The total alkaloid fraction of leaves found to be most potent. [75] The HT-29 cell line was the most sensitive fractions. The cytotoxic concentration (CTC50) values for all these fractions between 0.39-0.91, 0.68-2.8, 0.92-3.56, 4.05-8.2, 3.28-5.65 and 0.95-5.55μg/ml respectively for HT-29, RD-228, A-549, HEp-2, B-16, F-10 and Vero cell lines.

The six lignans including cyclolignan, 3, 4-dihydroxy cyclolignan were isolated from the flowering tops of *Larrea tridentate*. Additionally the flavone (4, 5-dihydroxy-7-methoxyflavanone) was isolated for the first time from *L.tridentata* or any member of the family Zygophyllaceae. All the compounds were assessed for their growth inhibitory activity against human breast cancer, human colon cancer and human melanomal cell lines. The lignans had IC_{50} values of 5-60 MICR Moles with their linear butane type lignans being the most potent. [76]

The daily administration of PBT-3 for 8 days to NU/NU mice bearing solid tumours derived from the s.c. administration of the leukemic cell line K562 results in inhibition of growth of the tumours *in vivo*, and this inhibition lasts for 60 days after stopping treatment with PBT-3 before recovery of tumour growth is re-established. Similar findings were observed when the mice were treated with Gleevec (STI-571). These results provide new evidence that PBT-3 is effective in controlling solid tumour growth *in vivo* and suggest that the PBT family may be useful in the development of new drugs in cancer therapy. [77]

The cytotoxic effects of antithymocyte globulin (ATG) in leukemic cells obtained from five patients with acute T lymphoblastic leukemia or precursor of T lymphoblastic leukemia have been studied by Ayami, Y. and his team. ATG itself killed leukemic cells in a dose-dependent manner. Simultaneous incubation with human AB or baby rabbit serum resulted in increased cytolysis of leukemic cells. These results suggested the presence of both direct and complement-mediated

cytolysis. Also it was examined for apoptotic cell death using Annexin-V. Cell incubation with ATG increased Annexin-V binding significantly compared with horse IgG (50.3±7.6% versus 95.7±1.8%, p = <0.0001). However, ATG did not induce apparent DNA fragmentation in a human T-ALL cell line. Neither anti-Fas MoAb (ZB4) nor a broad caspase inhibitor (z-VAD FMK) prevented this increase in Annexin-V binding. These results suggest that ATG induces leukemic cell death in a Fas/Fas-ligand- and caspase-independent manner. [78]

Treatment of human leukemic cell lines HL 60 and K562 with extracts of green and black tea and their polyphenols epigallocatechin and theaflavins respectively showed a dose dependent inhibition of growth as a result of cytotoxicity and suppression of cell proliferation. Flow cytometric analysis revealed the dose dependent increase in sub-G1 peak. The criteria confirmed the cytotoxic activity of green tea and black tea was found to be mediated through activation of caspase 3 and 8 particularly caspase 3 and by altering apoptosis related genes as evident by down regulation of Bcl-2 and up-regulation of Bax proteins. [79]

A formylated triterpene named cladocalol has been isolated from the leaves of *Eucalyptus cladocalyx* together with ursulolactone acetate, ursolicacid, 3β-acetate-12,20(29)-lupadien-28-oic acid, β-sitosterol and the known flavanoid eucalyptine were also isolated. Their structures were mainly established by extensive NMR studies (^{1}H NMR, DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC) and mass spectroscopy as well as by X-ray crystallographic analysis. In this study it is reported that Cladocalol has potent cytotoxic effect in myeloid leukemia cell line HL-60 by MTT assay. [80]

Actinonin causes inhibition of cellular proliferation in U937 Leukemia cells. Signs of apoptosis at high actinonin concentration included DNA fragmentation, exposure of phosphatidylserine and condensation of cell nuclei. The pathway of apoptosis was confirmed by the caspase activation. [81]

Sclareol is a labdane-type diterpene that has demonstrated a significant cytotoxic activity against human leukemic cell lines. The effect of sclareol against the human breast cancer cell lines MN1 and MDD2 derived from the parental cell line, MCF7. MN1 cells express functional p53, whereas MDD2 cells do not express p53. Flow cytometry analysis of the cell cycle indicated that sclareol was able to inhibit DNA synthesis induce arrest at the G0/1 phase of the cycle apoptosis independent of p53.[82] Sclareol-induced apoptosis was further assessed by detection of fragmented DNA in the cells. Furthermore, sclareol enhanced the activity of known anticancer drugs, doxorubicin, etoposide and cisplatinum, against MDD2 breast cancer cell line.

Three natural styryl lactones were isolated from *Goniothalamus griffithii* Hook and investigated their cytotoxicity on a panel of three hepatocyte cell lines, HepG2, drug resistant HepG2 (HepG2-R). All the three styryl lactones showed evident of cytotoxic activities on both HepG2 and HepG2-R cell lines; however, gonithalamin and goniodiol shows less toxicity on normal mice hepatocyte as the IC_{50} values of them on normal mice hepatocyte were about three times of that on HepG2. Morphological observation and cell cycle analysis were employed to elucidate the mechanisms of cytotoxicity of the tested compounds. Many apoptotic cells were observed in gonithalamin and altholactone-treated cells. Whereas, cells with chromosomes gathered at the equator were easily found in goniodiol-treated cultures. The analysis of cell cycle showed that G2/M arrest contributed to goniothalimin and gonidiol caused cell death and apoptosis was the cause of gonithalamin and altholactone induced cell death. The results suggested that the three styryl lactones may be prospectively developed into anti-tumor drugs, especially on treating drug-resistance tumor after structure modification. [83]

Four ent-kaurene diterpenes were isolated from the leaves of *Laetia thamnia* L: ent-kaur-16-en-19-oic acid (1a), ent-hydroxy-kaur-16-ene (2), ent-kaur-16-en-3a, 19-diol (3a), and ent-17-hydroxykaur-15-en-19-oic acid (4). The methyl ester (1b) of compound 1a and the acetate diester (3b) of compound 3a were prepared, and all compounds were evaluated for cytotoxicity against human prostate (22Rv1, LNCaP),

colon (HT29, HCT116, SW480, SW620) and breast (MCF-7) tumor cells at concentrations ranging from 6 to 50 mg/mL. The kaurenes showed activity in all cell lines tested. with the prostate cells demonstrating the most sensitivity as follows: 22 Rv1 cells towards 1a (IC50 5.03 mg/mL) and 1b (IC50 6.81 mg/mL), and LNCaP towards 2 (IC50 12.83 mg/mL) and 4 (IC50 17.63 mg/mL). [84]

Anti-proliferative and pro-apoptotic activities of fractions of *Pleurotus ostreatus* were examined using HT-29 colon cancer cells *in vitro*. A hot-water soluble fraction of the mycelium of the liquid cultured mushroom was partially isolated and chemically characterized as a low-molecular-weight glucan. HT-29 cells were exposed to the different isolates and significant inhibition of proliferation was obtained in a dose-dependent manner. Proliferation inhibition was shown to be the result of apoptotic induction because the pro-apoptotic molecules Bax and cytosolic cytochrome-c were up regulated. Fluorescence-activated cell sorter analyses of polysaccharide-treated HT-29 cells showed a high percentage of Annexin-positive cells. Here, a newly identified low-molecular-weight α-glucan has been described with promising anti-tumorigenic properties, and demonstrate its direct effect on colon cancer cell proliferation via induction of programmed cell death. [85]

Realgar (Arsenic containing Chinese traditional medicine) was shown to have a therapeutic effect against acute promyelocytic leukemia (APL) by inducing apoptosis. However, there is little data about the effects of it on plasma membrane. In a study, the cytotoxicity of realgar to HL-60 cells including its inhibiting cell growth, inducing apoptosis and bringing about membrane toxicity was investigated. It was suggested that realgar could significantly suppress the proliferation of HL-60 cells in a dose-dependent manner by 3-(4,5-dimethylthiazol-2-diphenyl-tetrazolium bromide (MTT) assay and the IC_{50} value was 5.67M. Flow cytometric analysis revealed that treatment with realgar resulted in increased percentages of apoptotic cells in a dose dependent manner. On the other hand, membrane lipid peroxidation level, lactate dehydrogenase (LDH) leakage and membrane surface topography alterations were investigated to assess the membrane toxicity induced by realgar. Treatment with

realgar at different concentrations accelerated membrane lipid peroxidation, potentiated LDH leakage, which was consistent with enhanced disorganization of membrane surface observed by atomic force microscopy (AFM). These results suggested that such membrane toxicity induced by realgar might play an important role in the process of apoptotic induction and could be considered as one of mechanisms underlying the cytotoxicity of realgar. [86]

Prostate cancer (PC) is the most prevalent cancer and the leading cause of male cancer death. *Azadirachta indica* (neem tree) has been used successfully centuries to reduce tumors by herbalists throughout Southeast Asia. Here the present study indicated that an ethanolic extract of neem has been shown to cause cell death of prostate cancer cells (PC-3) by inducing apoptosis as evidenced by a dose-dependent increase in DNA fragmentation and a decrease in cell viability. Western blot studies indicated that treatment with neem extract showed decreased level of Bcl-2, which is anti-apoptotic protein and increased the level of Bax protein. So the neem extract could be potentially effective against prostate cancer treatment. [87]

A carpin (3-hydroxy-9-methoxypterocarpan) (Medicarpin) and four isoflavones, 7-hydroxy-4-methoxy-isoflavone (Formononetin); 7,4-dimethoxyisoflavone; 5,4-dihydroxy-7-methoxy-isoflavone (Prunetin) and 7-hydroxy-6,4-dimethoxyisoflavone were isolated from the tuber roots of *Butea superba* Roxb. Compounds Formononetin and Prunetin showed moderate cytotoxic activity on KB cell lines with IC_{50} (M) values of 37.3±2.5 and 71.1±0.8 and on BC cell lines with IC_{50} (M) values of 32.7±1.5 and 47.3±0.3, respectively. [88]

The effect of trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, on the cell growth and apoptosis and its effect on the telomerase activity in human leukemic cell line U937 was carried out. Exposure of U937 cells to TSA resulted in growth inhibition and induction of apoptosis in a dose-dependent manner as measured by hemocytometer counts, fluorescence microscopy, agarose gel electrophoresis and flow cytometry analysis. The increase in apoptosis was associated with the up-

regulation in proapoptotic Bax expression and down-regulation of anti apoptotic Bcl-2 and Bcl-XL. TSA treatment inhibited the levels of cIAP family members and induced the proteolytic activation of caspase-3, which was associated with concomitant degradation of poly (ADP-ribose)-polymerase and β-catenin protein. TSA treatment markedly inhibited the activity of telomerase in a dose-dependent fashion. Additionally, the expression of human telomerase reverse transcriptase (hTERT), a main determinant of the telomerase enzymatic activity, was progressively down regulated by TSA treatment. It is therefore conclude that TSA demonstrated antiproliferative and apoptosis-inducing effects on U937 cells *in vitro*, and that changes in Bcl-2 family protein levels as well as telomerase activity may play an important role in its mechanism of action. [89]

2.5 Updated Perusal on Antioxidant Studies:

Oxidative free radical, which induced cellular damages have been implicated in many patho-physiological conditions like cancer, ageing process, neurodegenerative diseases, inflammation, diabetes mellitus, peptic ulcer etc. [90-91] Synovial fluid from the knee joints of human rheumatoid patients contains increased levels of diene conjugates and thiobarbiturates reactive substances (TBARS), suggestive of increased lipid peroxidation (LPO) *in vivo*. Superoxide dismutase (SOD) has well-established anti-inflammatory effect in several animal model systems of inflammation. They might directly scavenge such reactive oxidants as hydroxyl radical and hypochlorous acid. [92]

Free radicals not only oxidize the membrane lipids, but also can cause DNA damage that could lead to mutation. [93-94] Anti oxidant enzymes, like catalase and SOD levels are decreased during carcinogenesis. Oxidative stress has been suggested to play a key role as a mediator of apoptotic cell death in certain cell systems. [95]

Major natural defence system comprises oxidative free radical scavenging enzymes such as superoxide dismutase (SOD),[96] Glutathione peroxidase (GPX),[97] Lipid peroxidase (LPO),[98] reduced glutathione (GSH),[99] Catalase (CAT),[100] and

Glutathione-S-transferase (GST) [101] which can be estimated in liver and kidney homogenates. Activities of Aspartate transaminase(AST), alanine transaminase (ALT) [102] and urea [103] are estimated in serum.

Free radical scavenging activities of butanolic and methanolic extract of roots of *Pfaffia glomerata* was performed, the isolated constituent 20-hydroxyecdysone was also analysed for the same. 10 mg of each extract and the isolates were dissolved in 0.1% DMSO-PBS and screened for their capabilities on scavenging thiobarbituric acid reactive substances .The anti oxidant activity of each extract was determined *in vitro* by measuring malonyldialdehyde and 4-hydroxynonenal (4-HNE) in erythrocyte hosts treated with ferric ascorbate. [104]

Arthritin a poly herbal formulation was studied for the antioxidant activities. Poly herbal mixture have the extracts of *Accaia Arabica, Withania somnifera, Juniperus communis, Asparagus racemosus, Tinospora cordifolia, Tribulus terrestris, Anethum sowa, Curcumm zeruber and Zingiber officinalis.* In Freund's adjuvant induced arthritis , arthritin significantly reduces the enzyme LPO and increases the enzyme level of SOD, GPX and increased glutathione level.[105]

The antioxidant properties and total phenolic contents of two varieties of cowpea (*Vigna unguiculata*) were examined. The raw, dry heated and hydrothermal treated samples were extracted with 70% acetone and the extracts were freeze-dried. The unprocessed light brown seeds (LB) contained significantly higher level of total phenolics and tannins than the dark brown seeds (DB). The extracts were screened for their potential antioxidant activities using tests such as di-phenyl-picryl hydrazyl (DPPH), ABTS, FRAP, linoleic acid emulsion and beta-carotene-linoleic acid *in vitro* model systems. At 800 µg of extract in the reaction mixture, the superoxide anion radical scavenging activity was found to be significantly higher in the raw and dry heated seed extracts than the hydro thermally processed seed samples of the respective varieties. The DPPH radical and ABTS cation radical scavenging activities were well proved and correlated with the ferric reducing antioxidant capacity of the extracts. Interestingly, among the various extracts, dry heated samples of LB and DB

showed the highest hydroxyl radical scavenging activity of 83.6% and 68.2%, respectively. All extracts exhibited good antioxidant activity (74.3–84.6%) against the linoleic acid emulsion system. Using the β-carotene method, the values were significantly lower than BHT, BHA and Trolox. Owing to this property, the studies can be further extended to exploit not only the phenolic extracts but also the residual phenolic constituents associated with processed seed samples as health supplements and nutraceuticals. [106]

The antioxidant activity of methanol extracts of five plants from the genus Phyllanthus was evaluated by various antioxidant assays,[107] including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, reducing power and metal ion chelating activities. The various antioxidant activities were compared to standard antioxidants such as butylated hydroxytoluene and ascorbic acid. All the extracts showed strong antioxidant activity in all the tested methods. Among the five plants, *Phyllanthus debilis* has been found to possess the highest activity in all tested models. In addition to the antioxidant activity of these plants, the total phenolic compounds, flavonoids and flavonols were measured in the extracts. A correlation between the antioxidant activity and total phenolic content was observed.

The rhizome of the plant *Hypoxis rooperi* (''African potato'') is known for its traditional and ethno-medicinal uses in the treatment of benign prostatic hyperplasia and other diseases. Olga, L. *et al* have characterized an extract derived from *H. rooperi,* isolated its major bioactive compound, hypoxoside, and obtained its aglycone, rooperol, by enzymatic digestion. Absorption, fluorescence emission and bi dimensional NMR complete spectral data of these compounds were obtained. The antioxidant activity of both the compounds was fully analyzed through the thiobarbituric acid reactive substances (TBARS) and Trolox equivalent antioxidant capacity (TEAC) assays, and it was compared to catechins and olive biophenol. The study of the lipophilic and hydrophilic TEAC values revealed that more hydrophobic compounds had greater lipophilic TEAC values than the hydrophilic ones, indicating

that lipophilic TEAC assay may be more reliable for these compounds. The *H. rooperi* extract also showed higher antioxidant activity compared to other antioxidant herbal extracts, such as olive leaf or green tea. Moreover, neither evidence of acute oral toxicity nor adverse effects were observed when the *H. rooperi* commercial extract containing 45% hypoxoside was used at a dose of 2000 mg/kg. The results obtained in this work may contribute to understanding the biological activity described for these dicatechols and the African potato extract for food and cosmetic applications. [108]

Potential health effect of dietary exposure to environmental mercury was examined in a study by Xiuling, J. *et al.* Dietary exposure significantly increased content of reduced glutathione (GSH) and activity of glutathione peroxidase (GSH-Px) in rat liver at 7 or 20 days, but parameters droped to normal levels after 90 days of exposure. The early increases of the two antioxidants were partly associated with the co-cumulated selenium. However, activity of superoxide dismutase (SOD) was observed to be significantly decreased after 30 and 90 days of exposure. Changes of antioxidants were paralleled by the induction and aggravation of free radicals in rat liver at 30 and 90 days, increased nitric oxide (NO) content at 90 days . The excess availability of free radicals and the decreased levels of antioxidants resulted in a significant increase of malonyldialdehyde (MDA) after 90 days of exposure, indicating the aggravation of hepatic oxidative status. A number of biomarkers were required to monitor and minimize the health risk for the local population. [109]

The effects of milling on the phenolic content and antioxidant capacity of two wheat cultivars, namely CWAD (Canadian Western Amber Durum; *Triticum turgidum* L. var. durum) and CWRS (Canadian Western hard red spring; *Triticum aestivum* L.) were studied. The milling of wheat afforded several fractions, namely bran, flour, shorts and feed flour. In addition, semolina was the end product of durum wheat milling. Among different milling fractions the bran had the highest phenolic content while the endosperm possessed the lowest amount and this was also reflected in free radical and reactive oxygen species (ROS) scavenging capacity, reducing power and

iron (II) chelation capacity of different milling fractions in the two cultivars. This study demonstrated the importance of bran in the antioxidant activity of wheat, hence consumption of whole wheat grain may render beneficial health effects. [110]

Oxidative stress and oxidative damage to tissues are common end points of chronic diseases such as atherosclerosis, diabetes, and rheumatoid arthritis. Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can further increase the deleterious effects of free radicals. In a study the possible protective effects of *Murraya koenigii* leaves extract was evaluated against β-cell damage and antioxidant defense systems of plasma and pancreas in streptozotocin induced diabetes in rats. The levels of glucose and glycosylated hemoglobin in blood and insulin, Vitamin C, Vitamin E, ceruloplasmin, reduced glutathione and TBARS were estimated in plasma of control and experimental groups of rats. To assess the changes in the cellular antioxidant defense system such as the level of reduced glutathione and activities of superoxide dismutase, catalase and glutathione peroxidase were assayed in pancreatic tissue homogenate. The levels of glucose, glycosylated hemoglobin, insulin, TBARS, enzymatic and non-enzymatic antioxidants were altered in diabetic rats. These alterations were reverted back to near control levels after the treatment of *M. koenigii* leaves extract. Transmission electron microscopic studies also revealed the protective nature of *M. koenigii* leaves on pancreatic β cells. These findings suggested that *M. koenigii* treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress and pancreatic β cell damage. [111]

Methanol extracts of seven species of Indonesian seaweeds were evaluated for their antioxidant activity in a fish oil emulsion system. The system was incubated at 50°C for 3 and 24 h, in the presence of ferrous ion as a catalyst. Peroxide value (POV), ferrous ion chelating effect in the oil emulsion system and ferrous ion binding effect in methanol extracts were determined as oxidation markers. In the presence of ferrous ion catalyst, the entire methanol extracts from seaweeds showed significantly lower POV of the emulsion than the control, and the extract from *Caulerpa sertularoides* had the strongest antioxidant activity. The highest chelation on ferrous ion was also

found in the extract from *C. sertularoides* and it was significantly different compared to the other methanol extracts both in 3 and 24 h incubation. Methanol extracts of the seaweeds had excellent ferrous ion binding effect; however, their ability decreased in the fish oil emulsion system. [112]

Lipid peroxidation was carried out in the presence of the *S.verbenaca* hydromethanlic extract 10 and 100μg of extract/ml. $CuSo_4$ (10μM) was used as the oxidation initiator. Conjugated dienes (CD) formation ,oxygen consumption and thiobarbituric acid reactive substances(TBARS) formation were assessed to monitar the anti oxidant properties of the plant extract.[113] Butylated hydroxyl toluene (BHT) at 50μg/ml was used as a standard antioxidant. The hydromethanolic extract of *S.verbenaca* showed significant antioxidant effect at 100μg/ml concentration.

A study was undertaken to evaluate the anti-lipid peroxidative activity of an aqueous extract of *A.marmelos* fruits in streptozotocin deabetic rats in heart and pancreas. Oral administration of the extract for 30 days at the dose of 125,250 mg /kg produced a significant decrease in the elevated levels of peroxidation products viz, thiobarbituric acid reactive substances and hydroperoxides in the tissue of diabetic rats. Anti oxidant ezymes like catalase, SOD, GPX levels were increased significantly. [114]

The phytochemical investigation of hexane extract of *Iryanthera juruensis* fruits led to the isolation of two tocotrienols and four lignans, which exhibited antioxidant activity towards β carotene on TLC autographic assay. Two inactive quinines and three ω-arylalkanoic acids were also isolated .The isolates were investigated for the redox properties using cyclic voltammetry. The structure elucidation of the new compounds was based on analysis of spectroscopic data. [115]

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