Chapter 2

Review of Literature

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REVIEW OF LITERATURE

2.1. Phytochemicals

Flavonoids are large class of phenolics, exhibits antifungal (Chabot *et al*., 1992), anti-HIV (Pengsuparp *et al*., 1995; Critchfield *et al*., 1996), anti-inflammatory, hepato-protective, antithrombotic and anticarcinogenic activities (Middleton *et al*., 2000). The antioxidant properties of flavonoids are due to the functional hydroxyl groups present inhibiting the free radical formation, lowering redox potential and chelating the metals (Cook and Samman, 1996; Pietta, 2000); also they target microbial membrane. They are also reported for protecting membrane lipids from oxidation (Hossain *et al*., 2006).

Coumarins are active, phenolic compounds responsible for the characteristic odor of hay, possessing antithrombotic (Thastrup *et al*., 1985), anti-inflammatory (Piller, 1975), vasodilatory (Namba *et al*., 1988), anticoagulant (Keating and Kennedy, 1997) and antimicrobial activities. Catechins, the most reduced form of the C_3 unit in flavonoid compounds, inactivates cholera toxin in *Vibrio* (Borris, 1996) and inhibited isolated bacterial glucosyltransferases in mutans streptococci (Nakahara *et al*., 1993). Tannins are also a group of phenols, astringent in nature, protect the plants against decay and injury, possess antimicrobial activity (Jones *et al*., 1994).

Alkaloids are colorless, toxic to humans, aromatic in nature exhibiting anti-HIV (Sethi, 1979; Mcdevitt *et al*., 1996), antimalarial activities (Omulokoli *et al*., 1997) and they act by intercalation with DNA (Hopp *et al*., 1976; Phillipson and Neill, 1987). Terpenoids are widely distributed group found in essential oils and their derivatives posses various biological activities such as antibacterial (Thomson, 1978; Aureli *et al*., 1992), antifungal (Chaurasia and Vyas, 1977; Duke, 1985) and antimalarial (Vishwakarma, 1990) activities.

Carotenoids act as photoprotective agents, reducing skin related diseases, allergies, burns and cancer (Lee *et al*., 2000) due to their positive role on epithelisation process. Studies suggest that carotenoids may help to prevent prostate, breast (Cook *et al*., 1999), and skin cancer as well as endometrial cancer (Pelucchi *et al*., 2008). Astaxanthin, a carotenoid found in salmon, red fish, shrimps and crabs, exhibited anti-carcinogenic effects both *in vitro* and *in vivo* models by inducing apoptosis (Mou, 2005). Thus astaxanthin has been suggested as promising agent for use in chemoprevention or as a cancer therapeutic by Mou (2005).

2.2. *Mallotus* sp.

Earlier studies on *M. philippensis* fruits and bark evaluated for antioxidant activity displayed that bark possessed strongest activity (Arfan *et al*., 2007). *M. philippensis* methanolic bark extract showed presence of phenols and condensed tannins exhibiting high antioxidant activity (Arfan *et al*., 2009). *M. philippensis* yielded dimeric chalcone derivatives such as kamalachalcones A and B (Tanaka *et al*., 1998).

Phytochemical analysis of *M. peltatus* leaves showed the presence of tannin, triterpenoid, flavonoids, steroids, flavonoids, saponins and reducing sugars. The fractionation and purification yielded two fractions namely ursolic acid and b-sitosterol exhibiting significant anti-inflammatory, antioxidant, antibacterial and DNA protection activites (Arunachalam *et al*., 2009).

Stem and root bark of *M. repandus* yielded three different triterpenoids namely (1). 3a-hydroxy-13 a-ursan-28, 12b-olide 3-benzoate. (2). 3a-hydroxy-28b-methoxy-13a-ursan-28, 12b-epoxide 3-benzoate. (3). 3a-hydroxy-13 a-ursan-28-oic acid. Also four known compounds were acquired namely, 3-oxo-13a-ursan-28, 12b-olide; 3a-hydroxy-13a-ursan-28, 12b-olide; ursolic acid and bergenin (Huang *et al*., 1999).

Three new diterpenoids namely 10-hydroxy-cembrene-5-one, 6-hydroxy-cembrene-5,10-diene and 2a,4b,15,16-tetrahydroxyl-dolabradane were seperated from petroleum ether fractions of alcoholic extract of *Mallotus apelta* (Cheng and

Chen, 1999). Methanolic leaf extract of *M. apelta* yielded pentacyclic terpenoids namely 3a-hydroxyhop-22(29)-one, hennadiol, triedelin, epifriedelanol, taraxerone and epitaraxerol (Kiem *et al*., 2005). From *M. apelta* diterpenoids malloapeltine and 4, 5, 4'-trimethyl ellagic acid were isolated. The compound malloapeltine showed anti-HIV activity (Cheng *et al*., 1998).

M. japonicus pericarps yielded two phloroglucinol derivatives named mallotophenone and mallotochromene (Arisawa *et al*., 1985). Phloroglucinol derivatives of *M. japonicus* was found to suppress prostaglandin E-2 production by activated macrophages and also inhibit cytokine production (Ishii *et al*., 2002; 2003). *M. japonicus* contain bergenin, C-glucoside of 4-O-methyl gallic acid, reported for various bioactivities such as hepatoprotectivity, antioxidative, antiviral as well as antiulcerogenic and antiarthritic (Rastogi and Rawat, 2008). Six phenolic compounds namely mallotinic acid, mallotusinic acid, corilagin, geraniin, rutin and ellagic acid were seperated from *M. japonicus* exhibiting strong antioxidant activity (Tabata *et al*., 2010).

The whole plant of *M. metcalfianus* yielded six flavonoids including two new flavones, luteolin 7-o-(4"-o-(E)-coumaroyl)-b-glucopyranoside, chrysoriol-7-o-(4"-o-(E)-coumaroyl)-b-glucopyranoside and a mixture of flavonolignans, hydnocarpin7-o-(4"-o-(E)-coumaroyl)-b-glucopyranoside/hydnocarpin-D-7-o-(4"-o-(E)-coumaroyl)-b-glucopyranoside, exhibiting moderate antibacterial and good antioxidant activity (Riviere *et al*., 2009).

M. resinosus yielded simple coumarin, scopoletin which was reported to have DNA cleavage activity (Ma *et al*., 2004). From the leaves of *Mallotus pallidus*, phloroglucinal derivatives namely pallidusol, dehydropallidesol, pallidol, mallopallidol and homomallopallidol were isolated (Supudompol *et al*., 2004).

2.3. *Tabebuia sp.*

The family Bignoniaceae comprises of nearly 100 species of the genus *Tabebuia,* where six ones are common in Central America, 75 in West India and 25 in South America are reported (Haensel *et al*., 1994).

6-O-(p-coumaroyl)-catalpol, a specioside isolated from *T. rosea* bark failed to exhibit antibacterial activity but possessed antimalarial activity (Compadre *et al*., 1982). Also from the bark of *T. impetiginosa*, 19 glycosides such as 4 iridoid, 2 lignan, 2 isocoumarin, 3 phenylethanoid and 8 phenolic glycosides were isolated (Warashina *et al*., 2005). Further isolations yielded 12 compounds namely, 4 iridoid, phenyl ethanoid, 5 phenolic, lignin glucosides and seven known compounds (Warashina *et al*., 2006).

Three new compounds b-sitosterol, cyclolivil, lapachol and seven furonapthoquinones including two known 2-(1-hydroxyethyl)-naphtol(2,3-b)furan-4,9-quinone compounds were isolated from the trunkwood of *T. ochracea* (Zani *et al*., 1991). Inner stem bark of *Tabebuia ochracea* yielded five furanapthoquinones which were studied for antimalarial activity and exhibited high activity against *Plasmodium berghei* due to mixture of compounds (5- and 8-hydroxy-2-(1'-hydroxy)-ethyl-naphtho-[2,3-b]-furan-4,9-dione) 1 and 2, which couldn't be separated (Perez *et al*., 1997).

From the bark and trunkwood of *T. heptaphylla* previously described napthaquinone derivatives and known lignans such as cycloolivil, secoisolariciresinol and three lapachenol (lapachonone)- two napthofurans- a chromone and naphthalene derivatives were isolated (Hirschmann and Papastergion, 2003).

Two cyclopentane dialdehydes namely, 2-formyl - 5-(4'- methoxybenzoyloxy) – 3 – methyl – 2 – cyclopemtane – 1 - acetaldehyde and 2 - Formyl – 5 - (3', 4'- dimethoxybenzoyloxy) – 3 – methyl – 2 – cyclopentene – 1 - acetaldehyde were isolated from the bark of *T. impetiginosa* exhibited anti inflammotory activity (Koyama *et al*., 2000).

Also volatile constituents from dried inner bark of *Tabebuia impetiginosa* were extracted using steam distillation under reduced pressure followed by continuous liquid-liquid extraction. The major volatile constituents were 4-methoxybenzaldehyde (52.84 µg/g), 4-methoxyphenol (38.91 µg/g), 5-allyl-1,2,3-trimethoxybenzene (elemicin; 34.15 µg/g), 1-methoxy-4-(1E)-1-propenylbenzene

(trans-anethole; 33.75 µg/g), and 4-methoxybenzyl alcohol (30.29 µg/g). The antioxidant activities of the compounds were tested using two methods, conjugated diene and hexanal/hexanoic acid assays. The bark of *T. impetiginosa* extract appeared to be more active in hexanal/hexanoic acid assays than in the other assay when compared to a-tocopherol and BHT, also at a 12-fold higher concentration (125 vs 10 mg/ml), the bark of *T. impetiginosa* extract displayed 63 times less activity than a-tocopherol and 100 times less activity than BHT in conjugated assay (Park *et al*., 2003).

2-hydroxymethyl anthraquinone, anthraquinone-2-carboxylic acid and 2-hydroxy-3-(3-methyl-2- butenyl)-1,4-napthoquinone (lapachol) were isolated and identified from the inner bark of *T. impetiginosa* and studied for anti-*Helicobacter pylori* activity and the compound 2-hydroxymethyl anthraquinone displayed better activity (Park *et al*., 2006).

Tectol and a new novel dibenz-xanthene derivative named guayacanin were isolated from the heartwood of *T. guayacan* (Manners *et al*., 1975). Tecomaquinone III (quinone) was isolated from the heartwood of *T. pentaphylla* (Sharma *et al*., 1988). Lapachol, dehydro-a-lapachone, lapachenole, a and b-lapachone, dihydrolapachenole, nordihydrolapachenole, dehydrotectol and tetrahydrotectol and dimethyl ether, 1-hydroxy-2-methyl-and 2-hydroxy-3-methyl-anthraquinone compounds were seperated from the heartwood of *T. chrysantha* (Burnett and Thomson, 1968).

Extracts (34) from eight plant species of the Peruvian Amazonia currently used in traditional Peruvian medicine were tested for their antileishmanial, antitrypanosomal, and cytotoxic activity showed that hexane and chloroform extracts from *Cedrela odorata*, the alkaloid extract from *C. tomentosum* and the chloroform extract from *T. serratifolia* proved to be the most active against both parasites. Of the active compounds isolated from *T. serratifolia* naphthoquinones 2-acetyl-4H,9H-naphtho[2,3-b]furan-4,9-dione (2) and 2-(1-hydroxyethyl)-4H,9H-naphtho[2,3-b]furan-4,9-dione (3) showed 300 times more cytotoxic activity on CHO cell lines (Gonzalez-Coloma *et al*., 2012).

2.4. Antioxidant Activity of Plants

Iron is said to accelerate lipid peroxidation through fenton reaction (H_2O_2 + Fe_{2+} = Fe_{3+} + OH^- + OH^-) by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals leading to complete chain reaction (Halliwell, 1991). Medicinal plants are wealthy resources and is said to be reservoir of useful natural antioxidants which shield against oxidative tension and injuries resultant from lipid peroxidation (Repetto and Llesuy, 2002).

The flower, leaf, stem and seeds were analysed and hexane extract of flowers of *Hypericum scabrum* possessed significant antioxidant and antimicrobial activities due to the presence of omega-3 fatty acid (Shafaghat, 2011). The essential oil of *Geranium sanguineum* flowers displayed significant antioxidant activity (IC_{50}=85 µg/ml) and remarkable antibacterial activity against all test pathogens due to phenolic compounds (Hammami *et al*., 2011). The antioxidant activity (IC_{50}) of the *Wrightia tinctoria* flower extract was 43.16 µg/ml assessed by DPPH method and 124.07 mg AAE/100g of plant extract by phosphomolybednum method (Ramalakshmi *et al*., 2012a).

The antioxidant activity of *Pyrostegia venusta* flowers were said to be 95 % comparable with that of ascorbic acid (98.9 %) and BHT (97.6 %) (Roy *et al*., 2011). The antioxidant activity of oleander (*Nerium oleander*) flower showed significant activity (EC_{50}-2.11 ± 0.12) compared to standard ones, trolox (6.75 ± 0.22 µg/ml) and BHT (4.61 ± 1.61 µg/ml) (Ali *et al*., 2010). Four Indonesian medicinal plants screened for antioxidant activity displayed IC_{50} values (µg/ml) in the following order: *Phyllanthus niruri* (14.5), *Andrographis paniculata* (30.5), *Curcuma xanthorrhiza* (85.7) and *Curcuma aureginosa* (178.5) respectively (Nurcholis *et al*., 2012).

The IC_{50} values of essential oils of wild and cultivated varieties of *Thymus broussonetti, Thymus maroccanus* and *Thymus saturetoides* evidenced that wild and cultivated varieties of *Thymus maroccanus* (82.87; 88.42) were found to have higher antioxidants than *Thymus saturetoides* (167; 170.62) and *Thymus broussonetti* (132.33; 145.83) respectively (Bouzidia *et al*., 2013). The antioxidant activity (IC_{50})

of the *Mallotus tetracoccus* ethanolic bark extract was found to be 0.504 ± 0.002 µg/ml (Ramalakshmi and Muthuchelian, 2012b).

The methanolic extracts of three medicinal plants *Aloe ferox, Ptaeroxylon obliquum* and *Calpurnia aurea* demonstrated higher antioxidant activity (79.2 to 92.3 %) than water extracts at 0.2 mg/ml concentrations studied (Soyelu and Masika, 2012). The leaves of *Phlomis lanata*, *Nepeta melissifolia* and *Mentha pulegium* proved high antioxidant IC_{50} values of 23.9, 5.1 and 13.5 µg/ml respectively (Proestos *et al*., 2013). The petroleum ether, chloroform and methanolic fractionated stem bark extracts of *Moringa oleifera* extracts exhibited antioxidant IC_{50} values in the range of 124.75, 112.08 and 54.34 µg/ml (Kumbhare *et al*., 2012).

2.5. Antibacterial Activity of Plants

The methanolic extracts of *Casuarina equisetifolia, Cajanus cajan* and *Caesalpinia bonduc* displayed higher antibacterial activity with MIC values of 64, 128 and 128 µg/ml against *Staphylococcus aureus*, *Bacillus subtilis* and *Shigella sonnei* (Ahsan *et al*., 2009). The *Wrightia tinctoria* ethanolic flower extract showed highest inhibitions against *Vibrio chlorea* (16.76 ± 0.29 mm), *Escherichia coli* (14.02 ± 0.39 mm), *Staphylococcus aureus* (15.89 ± 0.41 mm) and *Klebsiella pneumonia* (10.2 ± 0.35 mm) (Ramalakshmi *et al*., 2012a).

A plenty of literature are emphasising that the root, leaf, stem and bark extracts of *Combrestatum adenogonium* exhibited high activity with minimum inhibitory concentration (MIC) values in range of 0.31 to 5 mg/ml (Mushi *et al*., 2012). In a study of fifty five plants, only four medicinal plants namely *Pinus monticola, Abies procera, Salvia vaseyi* and *Salvia apiana* proved high activity inhibiting *S. aureus*, and also 75 % non-medicinal plants revealed moderate bioactivity (Booth *et al*., 2012).

In another study of 52 Thai medicinal plants, highest activities were displayed by *Sonneratia alba, Sonneratia caseolaris* plants against *Pseudomonas aeruginosa* and *Candida albicans* and the ethanolic extracts of stems of *Coscinium fenestratum* and leaves of *Anacardium osccidentale* were also found to be more

effective (Kaewpiboon *et al*., 2012). Three Togolese medicinal plants *Balanites aegyptiaca, Entada africana* and *Parinari auratellifolia* revealed high MIC values in range of 0.90 to 1.8 mg/ml on *Serratia marcescens* but MIC value of 2.50 mg/ml against *E. coli, S. aureus and Proteus* (Karou *et al*., 2011).

Mallotus tetracoccus bark extract exhibited highest inhibitions in the order of *Klebsiella pneumonia* (25.33 ± 0.37 mm), *S. aureus* (22.83 ± 0.31 mm), *V. chlorea* (20.00 ± 0.31 mm) and *E. coli* (20.22 ± 0.26 mm) (Ramalakshmi and Muthuchelian, 2012b). The ethanolic and hexane fractions of Argentinan medicinal plant *Acacia aroma* displayed MIC values of 156 and 625 µg/ml on *S. aureus* and 78 and 1250 µg/ml of MIC values on *Listeria cytogenes* and bactericidal activity values of 312 and 5000 µg/ml respectively (Mattana *et al*., 2012).

2.6. Brine Shrimp Cytotoxicity of Plants

The cytotoxic potential (ED_{50}) of crude methanolic extract (CME), n-Hexane fraction (NHF) and aqueous fraction (AQF) of *Aster thomsonii* were studied, where AQF possessed maximum activity at 154.69 µg/ml (Bibi *et al*., 2011). Several other cytotoxicity studies displayed ED_{50} lesser than 1000 µg/ml for wild thyme, *Thymus serpyllum* (466 µg/ml) (Rehman *et al*., 2009) and out of 60 Brazilian medicinal plants screened for brine shrimp cytotoxic activity, only 10 % plants answered (Maria *et al*., 2000). Out of five Bangladesh medicinal plants studied for cytotoxicity, highest activities were detected for *Curcuma longa* (26.63 µg/ml), *Curcuma zedoaria* (145.87 µg/ml) and *Streblus asper* (224.74 µg/ml) (Akter *et al*., 2012).

The petroleum ether and methanolic seed extracts of *Khaya senegalensis* possessed significant LC_{50} values of 827.39 and 51.79 µg/ml (Juss *et al*., 2007). Investigations carried out on *Rubus imperialis* (Rosaceae) showed that methanol extracts and ethyl acetate fractions displayed good activity (Kanegusuku *et al*., 2001). *Wrightia tinctoria* ethanolic flower extract evidenced LC_{50} value of 3.54 µg/ml due to the presence of hexadecanoic acid, an ethyl ester reported for its various activities such as antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, haemolytic and 5- alpha reductase inhibitor activity

(Ramalakshmi *et al*., 2012a). Also about 226 methanol and water extracts of various plant species were screened for cytotoxicity, where only some were identified to have potential activity (Jacques *et al*., 2003).

Forty five medicinal plants of Kenya possessing antimalarial activity examined for cytotoxicity proved that only 23 (51 %) plants demonstrated activity at concentrations of less than 100 µg/ml, 18 (40 %) plants evidenced LC_{50} values between 100 to 500 µg/ml, 2 (4.5%) plants displayed LC_{50} values between 500 to 1000 µg/ml and 2 (4.5 %) plants confirmed LC_{50} values greater than 1000 µg/ml (Nguta *et al*., 2012). *Tabebuia rosea* flower extract proved LC_{50} value at 46.93 µg/ml when compared to taxol 0.85 µg/ml, due to the presence of flavonoids, phenols, esters and phenolic acids (Ramalakshmi *et al*., 2012c).

Thirty one medicinal plants of Eastern Nicaragua studied for cytotoxicity showed that only 4 (13 %) plants exhibited activity at concentrations < 1000 µg/ml, whereas 23 other (74 %) plants confirmed activity at concentrations of 1001 to 5000 µg/ml and 4 (13 %) at concentrations of 5001 to 7500 µg/ml (Coe *et al*., 2012).

From 4 of 7 extracts studied, leaf and stem extract of *Acer oblongifolium*, leaf and stem extract of *Hedera nepalensis*, leaf and flower extract of *Artimisia fragrans*, and root extract of *Artimisia fragrans* revealed significant ED_{50} values ranging from 11.9 to 226.8 ppm (< 1000 ppm). The highest rate of lethality to brine shrimp was noted in the case of leaf and flower extract of *Artemisia fragrans* (Inayatullah *et al*., 2007). *Mallotus tetracoccus* bark extract confirmed significant LC_{50} value at 84.72 µg/ml compared to taxol 0.85 µg/ml, due to flavonoids as major constituent exhibiting antitumor, antimicrobial, antioxidant and anti-inflammatory activities (Ramalakshmi and Muthuchelian, 2013).

2.7. Phytotoxicity of Plants

Rice (1984) has reported allelopathy as the harmful or beneficial effect by increasing or decreasing the associated plant growth in field environment through the release of allelochemicals (May and Ash, 1990). So for phytotoxic evaluation of plant extracts, radish seed phytotoxicity assay has evolved as a general prescreening

assay (Turker and Camper, 2002) measuring root length, shootlength and number of germinated seeds at different concentrations.

The isolated compounds ailanthone, ailanthinone, chaparrine, and ailanthinol B (quassinoid derivatives) of aqueous root extract of *Ailanthus altissima* studied for the allelopathic activity using radish, garden cress and purslane seeds; demonstrated that ailanthone had greatest inhibitory activity (Feo *et al*., 2003).

Kordali *et al*. (2008) clearly demonstrated that essential oil isolated from *Origanum acutidens* and their phenolic compounds namely carvacrol, thymol and p-cymene completely inhibited the growth of seedlings and roots also proved antifungal and insecticidal activities when compared to the standard compounds. The investigation on phytotoxic potential of some selected medicinal plants of the family Polygonaceae namely *Cenchrus ciliaris* and *Bothriochloa pertusa* (Hussain *et al*., 2010) evidenced significant allelopathic effects.

Turk *et al*. (2005) investigated the allelopathic effects of various black mustard (*Brassica nigra*) plant parts (leaf, stem, flower and root), where the extracts noteworthyly inhibited radish seed germination and seedling growth. *Tabebuia rosea* flower extract confirmed significant root length inhibition and seed germination inhibition at various concentrations studied, due to presence of alkaloids and phenolics compounds present in them (Ramalakshmi *et al*., 2012c). Of all the fractions of *Launaea procumbens,* water, methanolic and butanolic fractions exhibited marked growth inhibition of root and shoot while n-hexane and ethyl acetate fractions of both plants displayed moderate effects (Khan *et al*., 2011a).

2.8. Genotoxicity of Plants

The small micronuclei arising from predominately from broken fragments of chromosomes have been used as an index to test various physical and chemical toxic agents. Cytokinesis block micronucleus assay have been established and evolved for several years as a comprehensive method for measuring chromosome loss, breakage, non-disjunction, DNA misrepair, necrosis and apoptosis (Fenech, 2006).

The medicinal plants used to treat various diseases are checked for their genotoxic effects. The medicinal plants used for treating gastro intestinal disorders *Mouriri pusa, Qualea grandiflora* and *Qualea multiflora* confirmed an absence of mutagenicity (Santos *et al*., 2013). The ethanolic extract of the medicinal plant *Phyllanthus niruri* and Brazilian medicinal plant *Baccharis dracunculifolia* tested on lab mice and swiss mouse peripheral blood cells proved no clastogenic/genotoxic effects respectively (Andrade *et al*., 2008; Asare *et al*., 2012). Copaiba oil-resin extracted from trunk of *Copaifera* used in treating various disorders was tested in mice by comet assay and micronucleus assay proved no genotoxic or mutagenic effect (Almeida *et al*., 2012).

The essential oil of *Minthostachys verticillata* exhibited absence of genotoxicity on human PBMCs and erythrocytes of mice (Escobar *et al*., 2012). Ellagic acid naturally occurring and widely distributed plant phenol is said to demonstrate protective effect on mice cells tested (Berni *et al*., 2012). *Duguetia furfuracea* (Silva *et al*., 2012), *Calendula officinalis* (Leffa *et al*., 2012) and *Garcinia achachariae* (Marques *et al*., 2012) displayed no genotoxicity assessed by micronucleus test in mice.

2.9. Cytotoxicity of Medicinal Plants

Fifty five plants were studied for cytotoxic activity, where only 79 % revealed activity against cervical cancer cells (HeLa), of which 54 % proved high activity, 29 % exhibited mild activity and 17 % displayed no activity (Booth *et al*., 2012). The cytotoxicity of *Leea indica* leaves on three colon cancer cell lines exhibited IC_{50} greater than 100 µg/ml (Reddy *et al*., 2012).

In another study 52 Thai medicinal plants studied against human lung cancer (A549), breast cancer (MDA-MB-231), cervical cancer (KB3-1) and colon cancer (SW480) proved that only four plants confirmed effective cytotoxic activity namely vines of *Bauchinia strychnifolia*, stems of *Coscinium fenestratum,* roots of *Eurycoma longifolia* and leaves of *Kalanchao pinnata* possessing activities in the range of 1.2 to 3.25 µg/ml. Among the various solvent fractions used, the

dichloromethane and ethanol were said to be the most effective (Kaewpiboon *et al*., 2012).

Fractions I and III of two medicinal plants *Tephrosia purpurea* and *Ficus religiosa,* studied on MCF7 cell lines evidenced better activity in the range of 152.4 µM to 222.7 µM (Gulecha and Sivakumar, 2011). *Cephalotaxus griffithii* bark extracts on HeLa cancer cell lines demonstrated that the acetone fractions revealed IC_{50} value of 35.5 µg/ml (Moirangthem *et al*., 2012a). The cytotoxicity of Saudi Arabian plant *Conocarpus erectus* leaves, stems, fruits and flowers confirmed remarkable activities at concentrations less than 20 µg/ml on HepG2 and MCF-7 cell lines (Abdel-Hameed *et al*., 2012).

Ethnopharmacologically selected medicinal plants (33) from Democratic Republic of Congo were tested on MRC-5 cell lines, where only three medicinal plants namely stem bark of *Enantia chlorantha,* stem bark of *Piptadeniastrum africanum,* root bark of *Quassia africana* displayed IC_{50} activities lesser than 10 µg/ml (Muganza *et al*., 2012). The diethyl ether leaf extract *Sideritis scardica,* an endemic plant of Balkan Peninsula had dose dependent activity on B16 and Hl-60 cells at 100 µg/ml (Tadic *et al*., 2012).

Of the 102 species of Venezualan medicinal plants studied against six tumor cell lines, only seven plants *Clavija lancifolia, Hamelia pateus, Piper san-vicentense, Physalis cordata, Jacaranda copaia, Heliotropicum indicum* and *Annona squamosa* proved effective cytotoxicity (Taylor *et al*., 2013).

The IC_{50} values for α- mangostin and cycloart-24-en-3-β-ol compounds isolated from stem bark of *Garcinia malaccensis* were reported as 0.40 and 0.48 µM respectively on K562 cells (Taher *et al*., 2012). The ethanolic and hexane extracts of *Acacia aroma* on vero cell lines proved activity at 658 and 1020 µg/ml (Mattana *et al*., 2012). The petroleum ether fractions *Oxoxylum indicum* bark extracts on HeLa cancer cell lines exhibited IC_{50} value of 112.3 µg/ml, where the apoptotic activity was found at 33.2 % sub-G_0/G_1 (Moirangthem *et al*., 2013).

2.10. Silver Nanoparticles (AgNPs)

The plasmonic properties of silver nanoparticles are used as biosensors and as well as in imaging process for tumors. Silver nanoparticles biosensors can effectively biosense a large number of proteins such as in cancer disease diagnosis and are also widely used in thermal therapy in cancer for killing cancer cells (Loo *et al*., 2005). Silver nanoparticles are proved in wound healing by reducing pro-inflammatory cytokines, local matrix metalloproteinase (MMP) activities involved in inflammation (Kirsner *et al*., 2001), also inhibiting the activities of interferon gamma and tumor necrosis factor alpha involved in inflammation (Shin *et al*., 2007).

The mechanism of wound healing by silver nanoparticles have been due to the silver nanoparticles used as bone cements in combination with polymers such as polymethyl methacrylate have been applied for artificial joint replacements. Silver nanoparticle coated polypropylene mesh possess good antimicrobial activity, which can be considered an ideal candidate for surgical meshes (Cohen *et al*., 2007).

The silver nanoparticles prepared from various medicinal plant leaves extracts have been characterized and reported as follows: *Arbutus unedo* (Kouvaris *et al*., 2012), *Acyranthes aspera* (Venkatesh *et al*., 2013), *Malva parviflora* (Zayed *et al*., 2012), *Phyllanthus maderaspatensis* (Annamalai *et al*., 2012), *Aegle marmelos* (Rao and Paria, 2013a), *Ocimum sanctum* (Rao *et al*., 2013b), *Piper pedicellatum* (Tamuly *et al*., 2013), *Trachyspermum ammi* and *Papaver somniferum* (Vijayaraghavan *et al*., 2012), *Murraya koenigii* (Christensen *et al*., 2011), *Camellia sinensis* (Loo *et al*., 2012), *Panicum virgatum* (Mason *et al*., 2012), *Dalbergia sissoo* (Singh *et al*., 2012), and *Punica granatum* (peels) (Ahmad *et al*., 2012).

2.11. Cytotoxicity of Silver Nanoparticles

The silver nanoparticles prepared from various medicinal plant leaves extracts are reported for antioxidant, antifungal and hepatoprotective activities as follows: *Artemisia nilagirica* (Vijayakumar *et al*., 2013), *Cissus quadrangularis* (Sivakama valli and Vaseeharan, 2012), *Annona squamosa* (Jagtap and Bapat, 2012), *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini, Centella asiatica, Citrus sinensis* (Logeswari *et al*., 2012), *Terminalia chebula* (Kumar *et al*., 2012), *Altermisia capillaris* (Park *et al*., 2012), *Ocimum tenuiflorum* (Patil *et al*.,

2012), *Morinda citrifolia* (Satishkumar *et al*., 2012), *Cassia angustifolia* (Amaladhas *et al*., 2012), *Cleome viscose* (Sudhalakshmi, 2011), *Coleus aromaticus* (Vanaja and Annadurai, 2013) and root extract of *Trianthema decandra* (Geethalakshmi and Sarada, 2012) were accounted for high antimicrobial activities.

Also the silver nanoparticles are reported from various medicinal plant extracts of *Alternanthera sessilis* (Niraimathi *et al*., 2013), leaves of *Citrus lemon*) (Vankar and Shukla, 2012) and *Andrographis paniculata* (Suriyakalaa *et al*., 2013). The silver nanoparticles prepared from leaves of *Suaeda monoica* of 30 nm size revealed cytotoxicity on Hep-2 cell line with IC_{50} values of 300 nM (Satyavani *et al*., 2012).

The silver nanoparticles synthesized from *Cissus quadrangularis* showed particle size of 5-30 nm, exhibited antimicrobial and anticancer activity on Hep2 and Vero cell line with IC_{50} values of 64 µg and 90 µg respectively (Renugadevi *et al*., 2012). Nagajyothi *et al*. (2012) prepared silver nanoparticles using Chinese Quince fruit, *Pseudocydonia sinensis* which displayed antimicrobial and maximal cytotoxicity (81.85 %) to B16/F10 melanoma cancer cell line at concentration of 1 mM.

Subramanian and Suja (2012) prepared silver nanoparticles from *Coleus amboinicus* that exhibited antioxidant and cytotoxicity with IC_{50} value of 30 µg/ml on Ehrlich's ascite carcinoma cell line. The ethanolic extract of four medicinal plants namely *Phytolacca decandra, Gelsemium sempervirens, Hydrastis canadensis* and *Thuja occidentalis* used for silver nanoparticle synthesis evidenced significant antibacterial, antioxidant and cytotoxicity on skin melanoma cells (A375 cells) by G2/M phase cell cycle arrest (Das *et al*., 2013).

Recently the silver nanoparticles prepared from calli cells of *Citrullus colocynthis* (bitter cucumber) of 31 nm size proved cytotoxicity on Hep2 cell lines (human epideroid larynx carcinoma) causing cell death through apoptosis and DNA fragmentation (Satyavani *et al*., 2011). Recently egg white was used in conjugation with silver metal for Ag-protein bioconjugates preparation of 20 nm size, displayed biocompatibility on mouse fibroblast cell lines 3T3, where silver-protein bioconjugates enhanced the efficacy of irradiation (Lu *et al*., 2012).