CHAPTER 2

REVIEW OF LITERATURE

2.1 REVIEW OF PHARMACOLOGICAL AND PHYTOCHEMICAL STUDIES OF CEDRUS DEODARA LOUD.:

Awad et al., (2015) evaluated the probable mode of action of dihydroquercetin fraction of Cedrus deodara Loud. on immune status of gilthead sea bream. Cellular (phagocytosis and respiratory burst activities) and humoral (antiprotease, seric complement activity, peroxidase, total protein, bactericidal activity and IgM level) immune parameters were investigated. The fish received the lowest dose of dihydroquercetin showed a highly significant difference ($p < 0.05$) in phagocytosis, IgM level, respiratory burst, total protein, antiprotease and bactericidal activities compared to the control. Therefore, the results suggest that low concentrations of dihydroquercetin as food supplements are able to increase the immune status of gilthead sea bream.

Dhayabaran et al., (2014) isolated 3,4-bis(3,4-dimethoxyphenyl)furan-2,5-dione (BDFD) compound from the ethanolic extract of heart woods of Cedrus deodara Loud. and evaluated its anticonvulsant activity. The NMD induced lethality test and estimation of brain GABA were carried out to investigate the mechanism of action of the compound. BDFD showed dose dependent protection against pentylenetetrazole (PTZ), pilocarpine and 6-Hz-induced convulsions but it could not inhibit NMDA induced lethality. The therapeutic dose was reported to be below 100 mg/kg, with motor in-coordination at dose exceeded 400 mg/kg. Moreover, brain GABA estimation showed that this compound increases the GABA level. Whereas, BDFD showed a weak influence on the excitatory neurotransmitter glutamate.

Liang et al., (2014) isolated a novel compound viz. 2R, 3R-dihydromyricetin from the 50% methanol extract of pine needles of Cedrus deodara Loud. and studying its anti-
browning effect. The compound showed the potent monophenolase and diphenolase inhibitory activities. Moreover, they exhibited a strong free radical scavenging activity with a dose dependent manner. Its antibrowning effect was significantly better than ascorbic acid (0.5%) alone.

Zeng et al., (2014) isolated a novel antioxidant polysaccharide (APC) from pine needles of *Cedrus deodara* Loud. and evaluated its *in-vitro* antioxidant activity. APC was observed to be an acidic heteropolysaccharide and the backbone was mainly composed by glucose, mannose and xylose in the form of (1→4) linked. Meanwhile, APC exhibited the remarkable antioxidant activity to scavenge free radicals and inhibit the oxidative injury of DNA and cells.

Bai et al., (2013) studied the chemical constituents of the dichloromethane extract from pine needles of *Cedrus deodara* Loud. The chemical constituents were isolated and purified from the dichloromethane extract of pine needles by chromatography on silica gel and Sephadex LH-20. Seven compounds were isolated and their chemical structures were identified. Compounds ferulic acid, osthole, *beta*-phenylacrylic acid, paeonol, magnolol and honokiol were isolated from this plant for the first time.

Raza, et al., (2013) investigated the antioxidant potential of methanolic extracts of *Centella asiatica*, *Cedrus deodara* Loud. and *Artemisia persica* towards stabilization of sunflower oil as oxidation substrate. Results showed that all the plant extracts possessed antioxidant activity.

Emami et al., (2013) investigated the antioxidant activity of the extracts of leaves of six different species of Iranian common conifers including *Cedrus deodara* Loud. using ferric thiocyanate and thiobarbituric acid tests and compared with butylatedhydroxytoluene (BHT) and *alpha*-tocopherol. Results showed that methanol extracts of all the six species possessed antioxidant activity.

Zeng et al., (2012a) evaluated the antibacterial activity of water soluble extract from pine needles of *Cedrus deodara* Loud. on five food borne bacteria. Result showed that extract possesses a remarkable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus* and *Proteus vulgaris* with the minimum inhibitory concentration and minimum bactericidal concentration values in the ranges of 0.78-12.5 and 1.56-25 mg/ml respectively. They also isolated the shikimic acid from extract and reported their antibacterial activity.

Zeng et al., (2012b) evaluated the chemical structures, antioxidant and antimicrobial activities of essential oils from pine needles of *Cedrus deodara* Loud. Twenty three components, representing 95.79% of the oils were identified by GC-MS. The chief compounds include *alpha*-terpineol (30.2%), linalool (24.47%), limonene (17.01%), anethole
(14.57%), caryophyllene (3.14%) and eugenol (2.14%). The essential oils showed noteworthy antioxidant activity in scavenging free radicals. The essential oils also revealed strong antimicrobial activity against typical food borne microorganisms with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 0.2-1.56 and 0.39-6.25 μg/ml respectively.

Saab et al., (2012) evaluated the in-vitro antiproliferative activities of the wood essential oils of three Cedrus species (including Cedrus deodara Loud.) against K562 human chronic myelogenous leukaemia cells. The wood essential oils of Cedrus libani, Cedrus atlantica and Cedrus deodara Loud. inhibited the proliferation of the K562 cell line with IC50 values 23.38±1.7, 59.37±2.6 and 37.09±1.4 μg/ml respectively.

Chaudhary et al., (2012) isolated two new sesquiterpenes, (E)-(2S,3S,6R)-atlantone-2,3-diol and (E)-(2S,3S,6S)-atlantone-2,3,6-triol, along with two known sesquiterpenes, atlantolone and (E)-atlantone from Cedrus deodara Loud. The n-hexane and chloroform extracts of sawdust and atlantolone and (E)-atlantone exhibited antifungal activity against Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Aspergillus sydowii and Aspergillus ochraceus. The compound (E)-(2S,3S,6R)-atlantone-2,3-diol showed a weak antifungal activity against Aspergillus parasiticus and Aspergillus sydowii.

Kapoor and Sharma (2012) studied the efficacy of Himalayan cedar wood oils (Cedrus deodara Loud.) in rabbits which were naturally infested with Psoroptes cuniculi mites. Result showed that the mean lesion score in the Cedrus deodara Loud. oils treated group was significantly lower than the untreated group in the post treatment period and was comparable to benzyl benzoate emulsion treated group on days 14th and 21st post treatment.

Kurt and Isik (2012) compared the constituents of tars produced from woods of Cedrus libani by traditional methods and modern laboratory methods. In traditional methods of extraction, Taurus cedar tar contained 83 different components, 17 of which made up 86% of the compounds. The proportion of sesquiterpenoids in traditional process was 47.6%, whereas it ranged from 19% to 75% in studies based on laboratory extractions.

Maan et al., (2012) performed the pharmacognostical studies like macroscopical, microscopical and physical parameters like loss on drying, ash values and extractive values of the stem bark Cedrus deodara Loud. They also performed the various phytochemical tests on extracts and found that the stem bark showed the presence of flavonoids, tannins, proteins and amino acids in the different extracts. These studies provided referential information for correct identification and standardization of this plant material.

Dhayabaran et al., (2012) isolated BDFD from the ethanolic extract of heart wood of Cedrus deodara Loud. and investigated its anxiolytic activity using elevated plus maze,
open field test and light dark model in mice. The anxiolytic activity of BDFD showed promising results and could be useful for primary medical care.

**Liu et al., (2011a)** isolated two new myricetin glycosides, myricetin-3-O-(6″-O-E-p-coumaroyl)-α-D-glucopyranoside and 3′,5′-di-O-methylmyricetin-3-O-(6″-O-acetyl)-α-D-glucopyranoside, and three known flavonoids, myricetin, cedrin and 2R,3R-dihydromyricetin, from the pine needles of *Cedrus deodara* Loud.

**Liu et al., (2011b)** isolated and purified five flavonoids from ethyl acetate extract of pine needles of *Cedrus deodara* Loud. by chromatography. The structures were elucidated on the basis of spectroscopic analysis and chemical evidence. Five flavonoids were identified as cedrusone A, myricetin, 2R,3R-dihydromyricetin, quercetin and 2R,3R-dihydroquercetin.

**Liu et al., (2011c)** isolated and purified chemical constituents of flavonoids from ethyl acetate extract of pine needles of *Cedrus deodara* Loud. by chromatography. Five flavonoids were identified as 3′,5′-dimethoxymyricetin-3-O-(6″-O-acetyl)-α-D-glucopyranoside (1), myricetin (2), 2R,3R-dihydromyricetin (3), quercetin (4) and 2R,3R-dihydroquercetin (5). Compounds 2-5 are isolated from the pine needles in the plants of *Cedrus deodara* Loud. for the first time.

**Chaudhary et al., (2011)** isolated himachalenes and atlantones enriched fractions from wood chips of *Cedrus deodara* Loud. and forty compounds were identified from these fractions using GC and GC-MS. They also evaluated the insecticidal activity of oils and fractions against *Plutella xylostella* L. by using a leaf dip method. All fractions showed potential larvicidal activity but the himachalenes enriched fraction was more toxic (LC$_{50}$ = 362 μg/ml) than the atlantones enriched fraction (LC$_{50}$ = 365 μg/ml).

**Zaidi et al., (2011)** investigated the antiulcer and antiinflammatory activities of volatile oils (50 mg/kg) isolated from root of the plant *Cedrus deodara* Loud. The results showed that *Cedrus deodara* Loud. root oils has antiulcerative effects and may be used in the management of gastrointestinal disorders particularly in peptic ulcer.

**Dimri et al., (2011)** demonstrated the effectiveness of an indigenous preparation consisting of *Cedrus deodara* Loud. against dermatomycosis in goats, based on the total leukocyte count, total erythrocyte count, skin scraping examination, hemoglobin concentration, and changes in serum biochemistry including concentration of lipids, cholesterol, triglycerides, bilirubin, globulin and serum albumin. Other parameters like change in milk yield, body weight, physic chemical properties of meat and hepatic excretory function also responded positively to the treatment.

**Patil et al., (2011)** investigated the antihyperlipidemic effect of *Cedrus deodara* Loud. against monosodium glutamate (MSG) induced obesity in neonatal rats. Ethanolic and
acetone extracts at the age of 65 days showed significant reduction in body weight, serum glucose, total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein levels as compared to MSG control rats. Whereas, they significantly increased the body temperature, locomotors activity and high density lipoprotein level as compared to MSG control rats. They concluded that Cedrus deodara Loud. extracts exhibited antihyperlipidemic and antiobesity properties.

Zeng et al., (2011) evaluated the antibrowning and antibacterial activities of water soluble extract of pine needle of Cedrus deodara Loud. and related mechanism were also investigated by transmission electron microscope. Extract exhibited a strong antioxidant activity against free radicals with IC$_{50}$ of 25.5 ± 0.64 μg/ml. They also revealed that extract in combination with 0.5% ascorbic acid exhibited a synergistic antibrowning effect. Meanwhile, extract also showed a potent antimicrobial effect on all of the tested bacteria.

Dhayabaran et al., (2010) evaluated the anxiolytic and anticonvulsant activities of the alcoholic extract of heart wood of Cedrus deodara Loud. Anxiolytic activity was evaluated by using actophotometer, elevated plus maze and light-dark model. They found that extract reduced the aversion fear and produced anxiolytic activity in a dose dependent manner. The anticonvulsant activity was assessed by PTZ and maximal electro shock (MES) induced convulsions models in mice. The results showed that extract increased the onset of clonus and tonic seizures in PTZ induced convulsions model and decreased the duration of tonic extensor phase in MES induced convulsions model and also increased the percentage protection in PTZ and MES induced convulsions.

Paoli et al., (2011) isolated essential oils from wood of Cedrus atlantica growing in Corsica and were investigated by GC, GC-MS and $^{13}$C-NMR. Twenty three compounds accounting for 73.9-96.0% of the oils composition were identified. The oils consisted mainly of monoterpene hydrocarbons and sesquiterpenes, in particular α-pinene, himachalol, β-pi
ingenene, β-himachalene, γ-himachalene and α-himachalene.

Saxena et al., (2010) reported that natural antioxidants viz. curcumin, silymarin and acteoside, synergistically enhanced the anticancer potential of AP9-cd, a novel lignan composition from Cedrus deodara Loud. in human leukemia HL-60 cells. This study revealed that all four molecules behave as antioxidants.

Zhang et al., (2010a) isolated and elucidated the structure of one new phenyl propanoid, 1-[3-(4-hydroxyphenyl)-2-propenoate]-α-D-glucopyranosidea, along with nine known compounds viz. β-sitosterol, shikimic acid, 10-nonacosanol, dibutylphthalate, protocatechuic acid, phthalic acid bis-(2-ethylhexyl)ester, 5-p-trans-coumaroylquinic acid, ferulic acid β-D-glucoside, (++)-(6S,9R)-9-O-β-D-glucopyranosyloxy-6-hydroxy-3-oxo-α-ionol, all of which were obtained from ethanol extract of pine needles of Cedrus deodara Loud. for the first time.
Zhang et al., (2010b) investigated eight compounds from extract of *Cedrus deodara* Loud. viz. 9-hydroxy-dodecanoic acid, ethyl laurate, β-sitosterol, ethyl stearate, methylconiferin, 3-β-hydroxy-oleanolic acid methyl ester, ferulic acid β-glucoside and shikimic acid.

Parveen et al., (2010a) investigated the antifungal activity of *Cedrus deodara* Loud. root oils and isolated compounds against *Aspergillus fumigates* and *Candida albicans*. The root oils at the concentration of 150 μg/disc showed zone of inhibition against *Aspergillus fumigatus* but did not showed any activity against *Candida albicans*. The isolated compounds trans-atlantone and allo-himachalol did not showed any antifungal activity while, himachalol showed antifungal activity against *Aspergillus fumigates*.

Parveen et al., (2010b) evaluated the effect of *Cedrus deodara* Loud. root oils on the histopathology of the liver and kidney of Wistar albino rats. The oils were extracted from the plant root by dry destructive distillation method and administered orally at doses of 0.5 and 2.5 ml per rat. The root oils produced atrophic changes and haemolysis in the glomerular region as well as shrinkage and shedding of glomerular epithelium in the kidney tissues. Marked dilation of the central vein and congested blood vessels in the rat liver at both doses tested was also seen.

Devmurari (2010a) investigated antibacterial activity of the ethanolic extract of *Cedrus deodara* Loud. against three gram positive and three gram negative microbes and reported that they have a good antibacterial potentials.

Devmurari et al., (2010b) investigated the antihyperglycemic potential of ethanolic extract of *Cedrus deodara* Loud. wood at doses of 50 and 100 mg/kg body weight in alloxan induced hyperglycemic rats. The effect of ethanolic extract was estimated on 0 day, 5th day, 10th day and 14th day. A marked decrease in the blood sugar level was observed in hyperglycemic rats upon treatment. Preliminary qualitative chemical investigation showed the presence of alkaloids, glycosides, tannins, phenolic compounds, triterpenoids, fixed oils, fats and flavonoids. The results suggest that ethanolic extract has promising antihyperglycemic action in alloxan induced rats.

Ramesh et al., (2010) evaluated the diuretic and antiurolithiatic activities of petroleum ether extract of the heart wood of *Cedrus deodara* Loud. Concomitant administration of extract along with sodium oxalate prevented elevated serum biochemical levels due to the removal of these in urine. Histological study of the kidneys also showed that treatment had protected against sodium oxalate induced nephroliathiasis. These finding established the beneficiary effect of *Cedrus deodara* Loud. in urolithiasis.

Viswanatha et al., (2009) evaluated the anxiolytic and anticonvulsant activity of the alcoholic extract of heart wood of *Cedrus deodara* Loud. Elevated plus maze,
actophotometre and light dark model were used for testing anxiolytic activity. PTZ and MES induced convulsions models in mice were used for the assessment of anticonvulsant activity. The results suggest that alcoholic extract of heart wood of Cedrus deodara Loud. showed good anxiolytic and anticonvulsant activities.

Shivanand et al., (2009a) formulated the capsule formulation of ethanolic extract of Cedrus deodara Loud. wood and evaluated its physicochemical characterization and antidiabetic activity. The results of antidiabetic activity revealed that ethanolic extract had a vital role in the management of diabetes.

Saab et al., (2009) compared Lebanon's cedar with other kinds of Cedar's species. The essential oils, obtained by hydrodistillation from leaves of Cedrus libani and Cedrus deodara Loud. were analyzed by GC-MS system. Forty-nine components were identified from essential oils. The experimental data demonstrated that Germacrene D and β-caryophyllene were the main components in Cedrus libani while, benzaldehyde, myrcene and β-caryophyllene were the principal components of Cedrus deodara Loud.

Chaudhary et al., (2009) isolated the compounds from the essential oils and extract of the wood chips of Cedrus deodara Loud. After GC-FID and GC-MS analysis, thirty four compounds were identified from the essential oils and twenty six from the extract accounting for 98.3 and 94.6% of total identifications respectively. They reported that the major components of the oils were β-himachalene (38.3%), α-himachalene (17.1%) and γ-himachalene (12.6%) and from extracts were E-γ-atlantone (38.5%) and E-α-atlantone (10.2%).

Ahmad et al., (2008) reported antihyperglycemic effect of ethanolic extract of stem wood of plant Cedrus deodara Loud. in streptozotocin induced diabetic rats. Result showed that ethanolic extract exhibited significant fall in blood glucose profile in a single dose experiment on streptozotocin induced diabetic rats. Most likely it exerts multiple effects involving both pancreatic and extra pancreatic mechanism.

Yan-qiu et al., (2008) reported that volatile oils from Cedrus deodara Loud. leaves consisted 33 compounds out of which the α-pinene (24.98%), β-pinene (25.12%) and myrcene (13.10%) were the main constituents (63.20%).

Sachin et al., (2008) studied simultaneous HPLC determination of three bioactive constituents of Cedrus deodara Loud. namely wikstromol, matairesinol and dibenzylbutyrolactol and their pharmacokinetic profile in mice.

Sharma et al., (2008) investigated the mechanism of cell death in human cancer cells by AP9-cd, a synergistic lignan mixture from Cedrus deodara Loud. consisting of (-)-matairesinol, (-)-wikstromal and dibenzyl butyrolactol. The viability, morphological and ultra...
structural changes in Molt-4 cells was investigated. The light and electron microscopy identified the treatment induced loss in cell viability by activating the apoptotic process. AP9-cd causes the morphological changes of intracellular organelles in Molt-4 cells included the disruption of mitochondrial cristae, vacuolization, chromatin condensation and formation of micronuclei.

Parveen et al., (2008) investigated mammalian toxicity of Cedrus deodara Loud. root oils by oral administration against albino rats. The root oils of Cedrus deodara Loud. also have been investigated for sesquiterpenes and hydrocarbons by GC-mass analysis and spectral studies. They reported that root oils of Cedrus deodara Loud. is being used orally as antiulcer agent by Hakeems. The LD50 value of root oils was evaluated by probit mortality graph and was found to be 34.4 gm/kg. This is fairly safe as compared to Neem oils LD50 (5 gm/kg).

Singh et al., (2007) isolated a "CD lignan mixture" comprising lignans from stem wood of Cedrus deodara Loud. consisted of (-)-wikstromal (75-79%), (-)-matairesinol (9-13%) and benzylibutyrolactol (7-11%) and was studied for its cytotoxicity against human cancer cell lines. The in-vivo anticancer activity was studied using Ehrlich ascites carcinoma and colon carcinoma (CA-51) models in mice. The effects of CD lignan mixture on intracellular caspases, annexin V binding and DNA fragmentation were also studied to expand the mode of action. The results showed significant dose-dependent effects against several cancer cell lines from different tissues at 10, 30 and 100 µg/ml. This study revealed that CD lignan mixture has cytotoxic potential against human cancer cell lines.

Nisha et al., (2007) reported macrofilaricidal activity of methanolic extract of wood of Cedrus deodara Loud. The results of the worm motility assay showed that extract exhibited macrofilaricidal activity at concentrations below 4 mg/ml at an exposure period of 100 min with complete inhibition of worm motility and subsequent mortality was observed at 2 mg/ml. 3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide reduction assay was carried out at 1 mg/ml with 4h incubation period and the results showed that extract exhibited 86.56% inhibition in formazan formation compared to the control.

Shukla et al., (2006) reported that single, binary and tertiary combination of few plants derived molluscicides (including Cedrus deodara Loud.) alone or in combination with synergist caused a significant inhibition in different enzymes like acetyl cholinesterase (AChE), lactic dehydrogenase (LDH) and acid/alkaline phosphatase (ACP/ALP) in nervous tissue of frees water snail.

Bhushan et al., (2006) investigate the mechanism of cell death in human leukemia Molt-4 and HL-60 cells induced by AP9-cd, a standardized lignan composition from Cedrus deodara Loud. AP9-cd inhibited Molt-4 cell proliferation, increased sub-G0 cell fraction with no mitotic block, induced DNA ladder formation and produced apoptotic bodies. AP9-cd produced no cytotoxicity in primary rat hepatocyte culture at the concentrations used. AP9-cd
mediated early nitric oxide (NO) formation leads to caspases activation, mitochondrial depolarization and peroxide generation, which may be accountable for mitochondrial dependent and independent apoptotic pathways involved in the killing of leukemia cells by AP9-cd.

Makhaik et al., (2005) identified three major component viz. himachalene (59%), cis-atlantone and α-atlantone (19%) in essential oils of Cedrus deodara Loud. wood chips and reported their antimosquito properties against C. quinquefasciatus and A. aegypti.

Barrero et al., (2005) isolated five abietanes diterpenoids from neutral part of the hexane extract of the cone of Cedrus atlantica. viz. 9α, 13α-epidioxiabiet-8(14)-en-18-ol, 7α, 18-diacetox, 9β, 13β-epidioxiabiet-8(14)-ene, 7α, 18-diacetoxyabiet-8(14)-ene-13β-ol, 7α, 18-diacetoxy-13β-methoxyabiet-8(14)-ene and 13β-hydroxyabiet-8(14)-en-7-one.

Rao et al., (2003b) reported the effect of single and binary treatments of plant derived molluscicides on different enzymes like AChE, LDH and ACP/ALP in the nervous tissue of the harmful terrestrial snail Achatina fulica. The results showed that binary treatment of Allium sativum bulb powder + Cedrus deodara Loud. oils were more effective against AChE, ALP and LDH than the single treatment.

Thabrew et al., (2003) reported antiinflammatory and analgesic potential of Maharasnadhi Quathar (MRQ, containing Cedrus deodara Loud.), a polyherbal preparation recommended by Ayurvedic medical practitioners for treatment of arthritic conditions. Results showed that MRQ significantly and dose dependently inhibited carrageenan induced rat paw edema indicating its antiinflammatory potential. They also increased the reaction time of rats in the hot plate test but it had no effect on the reaction time in the tail flick test, these results indicated that MRQ possesses analgesic activity that is probably mediated via a supra spinal effect.

Thabrew et al., (2001) reported antioxidant potential of two polyherbal preparations viz. maharasnadi quathar (MRQ, contains Cedrus deodara Loud.) and weldehi choornaya (WC) used in Ayurveda for the treatment of rheumatoid arthritis. Antioxidant potentials of these preparations were assessed by their effects in rheumatoid arthritis patients on: (a) activities of the enzymes SOD, glutathione peroxidase and catalase; (b) lipid peroxidation (as estimated by thiobarbituric acid reacting substances, TBARS) and (c) concentrations of serum iron, haemoglobin and the total iron binding capacity. The results of the study demonstrated that MRQ has much greater antioxidant potential than WC.

Tiwari et al., (2001) performed an activity-directed fractionation and purification process to identify the antioxidant components of Cedrus deodara Loud. The results showed that the chloroform extract has strong antioxidant activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. They isolated three compounds with potent antioxidant activity and
identified by spectroscopic methods (\(^{1}\)H NMR, \(^{13}\)C NMR, IR and MS) as (-)-matairesinol, (-)-nortrachelogenin and dibenzylbutyrolactollignan (4, 4', 9-trihydroxy-3, 3'-dimethoxy-9, 9'-epoxylignan).

**Srivastava and Kulshestra (2001)** isolated one new diterpene acid with abietane skeleton, designed as centdaroic acid beside seven other known compounds viz. \(\alpha\)-atlantone, allohimachlol, himachalol, \(\alpha\)-amyrin acetate, oleanolic acid, lawsatitol, and centdarol from \(n\)-hexane soluble fraction of ethanolic extract of root of *Cedrus deodara* Loud.

**Rao and Singh (2001)** reported the toxic effects of the binary and tertiary combination of plants derived molluscicides (*Azadirachta indica* and *Cedrus deodara* Loud. oils) with synergistic (MGK-264, piperonyl butoxide and fruit powder of *Embelia ribes*) against the *Lymnaea accuminata*. The binary and tertiary mixtures of plants derived molluscicides with *synergistic* were more toxic with respect to the single treatment of plants derived molluscicides. They found maximum synergistic action in binary and tertiary combination in *Azadirachta indica* and *Cedrus deodara* Loud. and *Azadirachta indica*, piperonyl butoxide and *Cedrus deodara* Loud. in 1:7 and 1:5:7 ratio respectively.

**Shinde et al., (1999a)** reported the antiinflammatory and analgesic activities of volatile oils of *Cedrus deodara* Loud. wood at the doses of 50 and 100 mg/kg body weight. The oils produced significant inhibition of carrageenan induced rat paw edema, exudative proliferative and chronic phases of inflammation in adjuvant arthritic rats at both doses. The volatile oils at both tested doses were also found to possess analgesic activity against acetic acid induced writhing and hot plate reaction in mice.

**Shinde et al., (1999b)** reported membrane stabilizing activity and possible mechanism of action for the antiinflammatory activity of volatile oils of *Cedrus deodara* Loud.

**Shinde et al., (1999c)** reported preliminary studies on the immunomodulatory activity of volatile oils of *Cedrus deodara* Loud.


**Singh and Singh (1998)** reported the molluscicides activity of the essential oils of *Cedrus deodara* Roxb. and neem tree, powder from bulbs of garlic and oleoresin extracted from rhizomes of ginger in different combination. Results showed that a mixture of cedar and neem oils were most toxic to *Lymnaea accuminata* of the combination tested.

**Sharma et al., (1997)** reported the efficacy of oils of *Cedrus deodara* Loud. in controlling sarcoptic mange in 24 lambs (3-6 months) naturally infected with *Sarcoptes mites.*
They found that animals treated with oils of *Cedrus deodara* Loud. had significantly more erythrocyte and leukocyte counts compared to control. Oils of *Cedrus deodara* Loud. was found more efficacious in controlling sarcotic mange in sheep.

**Khan and Naheed (1990)** reported that petroleum ether soluble fraction of *Cedrus deodara* Loud. bark has a large number of hydrocarbons. They isolated and characterized the saturated straight and branched chain hydrocarbons ranging from C$_{14}$-C$_{20}$ and unsaturated sesquiterpene hydrocarbons with empirical formula C$_{15}$H$_{22}$. They found four known isomers of himachalene with one new isomer with double bonds in conjugated position 4 and 10.

**Tondan et al., (1989)** reported the safety profile of *Cedrus deodara* Loud. and found that it was non-toxic, non-irritant to the skin of rabbit and sheep and they did not alter blood urea nitrogen and blood glucose levels.

**Singh and Agarwal (1988)** isolated himchalol (3%) and 3-himachalene (31%) from Himalayan cedar wood oils. The activity of wood oils and isolated compounds were assessed against the pulse beetle (*Callosobruchus analis* F.) and the housefly (*Musca domestica* L.). Results showed that himachalol produced the highest mortality with quick effect against both test species, while similar activity of 3-himachalene was observed against the pulse beetle only.

**Ohmoto et al., (1987a)** isolated five known compounds and two new compounds from the ether extract of pollen grain of *Cedrus deodara* Loud. The known compounds were identified as naringenin, dehydroabietic acid, β-sitosteryl β-D-glucoside, 15-hydroxy-dehydroabietic acid and 7α, 18-dihydroxydehydroabietanol. The new compounds were characterized as hexadecane-1, 16-diol 7-caffeoyl ester and 7β, 15-dihydroxydehydroabietic acid.

**Ohmoto et al., (1987b)** isolated three new compounds from the pollen grain of *Cedrus deodara* Loud. and these compounds were identified as 15-methoxy-abietic acid, 9-caffeoyloxyhexadecanol and 7β, 18-dihydroxydehydroabietanol. They also isolated two known compounds namely 15-hydroxy abietic acid and 7β-hydroxydehydro abietic acid.

**Ibata et al., (1984)** isolated polyprenols with an average number of isoprene residues of 15 to 18 from the needles of six plants in the Pinaceae with content of 0.2-2.0% of the dry weight. $^1$H NMR, $^{13}$C NMR and FDMS spectroscopy revealed that all of the polyprenols were long chain homologues of betulaprenols.

**Bhan et al., (1984)** isolated one known compound $\Delta^{10}$-dehydroepitodomatuic acid and two new related compounds characterized as $\Delta^7$-dehydrotodomatuic acid and 7-hydroxytodomatuic acid from the wood of *Cedrus deodara* Loud. Moreover, minor amounts
of geronic acid, limonene-8-carboxylic acid and 4-acetylcyclohex-l-ene-l-carboxylic acid were also isolated.

**Agrawal and Rastogi (1982)** isolated two new lignans from the lead acetate purified butanol soluble fraction of *Cedrus deodara* Loud. wood and were identified as benzofuranoid neolignan and 1, 4-diarylbutane lignin.

**Agrawal and Rastogi (1981)** isolated a novel type of phenolic sesquiterpenes himasecolone and a known compound isopimaric acid from the chloroform soluble fraction of wood extract of *Cedrus deodara* Loud.

**Agrawal et al., (1980)** identified the taxifolin and elucidated the structure of cedrin (6-methyldihydmomericin), cedeodarin (6-methyltaxifolin), cedrinoside and dihydromyricetin from cedar wood.

**Krishnappa and Dev (1978)** isolated and elucidated the structure of a C11 monocarboxylic acid (limonene carboxylic acid), a nor-sesquiterpene and a sesquiterpene diosphenol (Deodardione) from the essential oils of *Cedrus deodara* Loud.

**Dikshit et al., (1978)** proved the fungicidal activity of *Cedrus* oils and were found more effective than the synthetic fungicides like copper oxychloride, mancozeb, phenylmercuricacetate and wettable sulphur.

**Shankaranarayan et al., (1977a)** reported the isolation and structure elucidation of a new bicyclic sesquiterpene (isohimachalone) from the essential oils of wood of *Cedrus deodara* Loud.

**Shankaranarayan et al., (1977b)** isolate and elucidated the structure of oxidohimachalene, a minor constituent of the essential oils of *Cedrus deodara* Loud. A biomimetic type conversion of β-himachalene into oxidohimachalene employing photochemical hydroxylation or "copper peroxide" oxygenation was also reported.

**Shankaranarayan et al., (1977c)** isolated and elucidated the structure of a novel bisabolane based tetrahydro-γ-pyrone from the essential oils of *Cedrus deodara* Loud. The isolated compound characterized as deodorone has been chemically correlated with the sesquiterpene ketone, atlantone. Moreover, a hydroxy ketone characterized as atlantolone has also been isolated.

**Kulshreshtha and Rastogi (1976)** isolated the isocentdarol, a sesquiterpenediol constitution from *Cedrus deodara* Loud.
Kar et al., (1975) identified himachalol as the major antispasmodic constituent in the wood of Cedrus deodara Loud. The pharmacological studies of himachalol on various isolated smooth muscles and against different agonists (acetylcholine, serotonin, histamine, barium chloride and nicotine) indicated spasmolytic activity similar to that of papaverine. Intravenous injection of himachalol at doses of 3-10 mg/kg in the cat produced a dose dependent fall in blood pressure and an increased femoral blood flow.

Shankaranarayanan et al., (1973) isolated deodarone, a novel sesquiterpene tetra hydro-γ-pyrones from essential oils of Cedrus deodara Loud.

Pande et al., (1971) isolated and characterized pure atlantone for the first time from essential oils of Cedrus deodara Loud. They also reported that atlantone was found in both major trans- and minor cis- geometry.

Bisarya and Dev (1968a) described the isolation and structure determination of himachalol, a constituent of the essential oils of Cedrus deodara Loud. This alcohol was directly related to the recently described himachalenes.

Bisarya and Dev (1968b) isolated allohimachalol, a sesquiterpenes alcohol from the essential oils of Cedrus deodara Loud.

Joseph and Dev (1968) isolated α- and β-himachalenes, the two major sesquiterpene components of the essential oils of Himalayan deodar.

Adinarayana and Seshadri (1965) isolated a new dihydroflavonol named deodarin (3', 4', 5, 6-tetrahydroxy-8-methyl dihydroflavonol) from the stem bark of Cedrus deodara Loud.

2.2 REVIEW OF PHARMACOLOGICAL AND PHYTOCHEMICAL STUDIES OF PINUS ROXBURGHII SARG.: 

Kaushik et al., (2014) established a relationship between ethnopharmacological claims and bioactive constituents present in Pinus roxburghii Sarg. against all possible targets for diabetes through molecular docking and to develop a pharmacophore model for the active target. The process of molecular docking involved study of different bonding modes of one ligand with active cavities of target receptors protein tyrosine phosphatase 1-beta, dipeptidyl peptidase-IV, aldose reductase and insulin receptor with help of docking software Molegro virtual docker. From the results it was observed that secoisoresinol, cedodarin and pinoresinol showed the utmost docking results on almost all the receptors.
Shuaib et al., (2014) isolated new abietatriene type diterpenes linked with lanostenes from oleo-resin of Pinus roxburghii Sarg. The isolated compounds were identified as pinusquinoic acid, 12-hydroxydehydroabietic acid, pinusoic acid A, B and C.

Kaushik et al., (2013) reported traditional uses of Pinus roxburghii Sarg. in traditional and folkloric systems of medicine. They reported that all parts of this plant possess medicinal qualities in Ayurvedic and Unani systems of medicine. They reported the use of this plant for healing of many diseases of blood, skin, eyes, ears and throat. They also reported that this plant parts are rich in various bioactive compounds such as α-pinene, quercetin, abietic acid and xanthone. Resin acids and flavonoids form a major portion of these bioactive compounds.

Cretu et al., (2013) studied on crude hydromethanolic extract of Pinus brutia bark and its fractions (diethyl ether, n-butanol, ethyl acetate and aqueous) with regard to their phenolic content and antioxidant activities. The results showed that bark extract had high phenolic contents. They identified taxifolin in diethyl ether extract and catechin, taxifolin-O-hexoside and procyanidin in ethyl acetate extract. They also reported the significant antioxidant activities of all extracts except diethyl ether extract. They conclude that Pinus brutia bark is very promising for the dietary supplements industry due to its high free radical scavenging and 15-lipoxygenase inhibitory effects.

Shuaib et al., (2013) studied the in-vitro antibacterial activity of resin rich methanolic extract of Pinus roxburghii Sarg. by agar-well diffusion method against gram-positive and gram-negative bacterial strains. The results showed that among all the bacterial strains tested, Enterococcus faecalis was found most sensitive and Salmonella typhi was resistant to Pinus roxburghii.

Satyal et al., (2012) examined the antimicrobial and cytotoxic activities of the essential oils of cone, needle and bark of Pinus roxburghii Sarg. GC-MS analysis revealed that essential oils were dominated by sesquiterpenes, mainly (E)-caryophyllene and α-humulene as well as monoterpenic alcohols, terpinen-4-ol and α-terpineol. The monoterpenic δ-3-carene was present only in needle and cone essential oils. Bio-activity assays of the cone essential oils showed remarkable cytotoxic activity (100 % killing of MCF-7 cells at 100 μg/mL) along with notable brine shrimp lethality (LC_50 = 11.8 μg/mL). However, the cone essential oils didn’t showed antibacterial activity but exhibited antifungal activity against Aspergillus niger.

Kaushik et al., (2012a) reported the analgesic and anti-inflammatory activities of alcoholic extract of Pinus roxburghii Sarg. Analgesic activity was evaluated by acetic acid induced writhing and tail immersion tests in Swiss albino mice whereas, antiinflammatory activity was evaluated by carrageenan induced paw oedema and cotton pellet granuloma in Wistar albino rats. They found that alcoholic extract of bark exhibited significant analgesic and antiinflammatory activities in the tested models.
Kaushik et al., (2012b) reported the anticonvulsant activity of alcoholic extract of Pinus roxburghii Sarg. bark by MES and PTZ induced seizure in rats. In MES model, alcoholic extract reduced all phases of convulsion significantly. In PTZ model, the administration of the extract 30 min prior to injection of PTZ significantly delayed the onset of clonic seizure.

Sharma et al., (2012) reported the uses of resin extracted from the stem of Pinus roxburghii Sarg. for preventing infection of broken horn and removing external parasites. They reported it’s used as ethno veterinary remedies of diseases among milk yielding animals in Jammu and Kashmir.

Maimoona et al., (2011) estimated total flavonoids and phenolic content quantitatively by using colorimetric method in various fractions of bark and needle extracts of Pinus roxburghii Sarg. and Pinus wallichiana. For the measurement of total flavonoids content the plant extracts were hydrolyzed and aglycone was measured before and after hydrolysis to calculate the presence of free aglycone content. They found that all fractions of the bark and needles of two pines, with the exception of aqueous fraction, contain flavonoids and phenolics to a varying extent.

Puri et al., (2011) studied the antidyslipidemic and antioxidant activities of methanolic extract of needle of Pinus roxburghii Sarg. Results showed that methanolic extract (100 mg/kg) significantly decreased the plasma triglyceride by 38%, total cholesterol by 23%, glycerol by 44% and low density lipoproteins cholesterol by 27% accompanied with increase in high density lipoproteins cholesterol. Antioxidant activity of fractions and alcoholic extract was assessed by Trolox equivalent antioxidant capacity assay, and results showed that alcoholic extract and n-butanol insoluble fraction have significant antioxidant capacity.

Zafar et al., (2010) identified nine major components in essential oils of Pinus roxburghii Sarg. needles and were recognized as α-pinene (29.3%), caryophyllene (21.9%), 3-carene (14.2%), (10.5%), α-terpineol (4.5%), caryophyllene oxide (3.1%), borneol acetate (2.2%), α-longipinene (1.2%), β-myrecene (1.1%) and terpinyl acetate (1.0%).

Abbasi et al., (2010) reported ethno pharmacological applications of medicinal plants including Pinus roxburghii Sarg. to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. They reported that juice of fresh leaves of Pinus roxburghii Sarg. is taken orally for the treatment of measles and slightly wormed resin is applied directly for the treatment of boils and warts.

Kunwar et al., (2009) reported indigenous uses and ethno pharmacology of medicinal plants including Pinus roxburghii Sarg. in Far-west Nepal. These medicinal plants were reported to help in alleviating human suffering and are widely used for subsistence, home remedies, and trade. It is estimated that 70-80% of people worldwide rely on traditional
herbal medicine to meet their primary health care needs. They also reported the use of wood oils of *Pinus roxburghii* Sarg. as nerve tonic, diuretic, haemostatic, expectorant and for management of ulcer, burns, skin diseases and cracks.

Hassan and Amjid (2009) studied on GC-MS of essential oils of *Pinus roxburghii* Sarg. stem and reported preliminary antibacterial and antifungal activities on some microorganisms.

Rehman and Iqbal (2009) analyzed essential oils of *Pinus roxburghii* Sarg. stems by GC-MS and performed its antimicrobial activity. They reported pinene, 3-carene, caryophyllene, terpinenol, pcyrne, limonene, phallenderene, farnesyle acetate, borneol acetate, caryophyllene oxide, camphene, butanoic acid, tepinyl acetate, o-cymene, 3-methyl-, 2-phenylethyl ester,1-terpinen-4-ol, and terpinene were the major components of essential oils. They observed antibacterial activity of essential oils against *Staphylococcus aureus* and *Bacillus subtilis*, while no activity were observed against *Escherichia coli* and *Enterobacter aerogenes*. They also reported the antifungal activity of essential oils against *Aspergillus niger*, *Aspergillus terrus*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus vescicolor* and *Trichoderma viride*.

Farooq et al., (2008) identified the traditional healers and documented the ethno veterinary medicinal practices of eighteen plants including *Pinus roxburghii* Sarg. for the treatment of different parasitic diseases of livestock in Cholistan desert, Pakistan.

Bissa et al., (2008) reported antibacterial potency of petroleum ether, ethanol, chloroform and water extracts of aerial parts of three naked seeded plants including *Pinus roxburghii* Sarg. against four human pathogenic bacteria (*Salmonella typhi*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*) and one plant pathogenic bacteria (*Agrobacterium tumefaciens*).

Nemova et al., (2007) reported antiinflammatory and analgesic uses of Rumalaya, a complex plant preparation (including *Pinus roxburghii* Sarg.) produced by Himalaya Drug Company.

Uniyal et al., (2006) reported traditional uses of medicinal plants including *Pinus roxburghii* Sarg. for curing various ailments among the tribal communities of Chhota Bhangal, Western Himalaya. They reported that the extract of *Pinus roxburghii* Sarg. was taken to increase the flow of urine and used as diuretic.

Hussain et al., (2006) reported traditional, medicinal and economic uses of gymnosperms of Kaghan Valley, Pakistan. They reported that wood of *Pinus roxburghii* Sarg. is diaphoretic and stimulant and used in burning of body, cough, fainting, ulceration, snake bite and scorpion sting.
Kumar et al., (2006) reported that *Pinus gerardiana* Wall. (Pinaceae) is used as wound healing, antibacterial and antifungal agents in Indian system of medicine.

Makhaik et al., (2005) reported antimosquito properties of essential oils from *Cedrus deodara*, *Eucalyptus citriodara*, *Cymbopogon flexuous*, *Pinus roxburghii* Sarg. *Syzygium aromaticum*, *Tagetes minuta* against the adult *Culex quinquefasciatus* and *Aedes aegypti*.

Chopra et al., (2002) reported the traditional uses of *Pinus roxburghii* Sarg. They reported that turpentine obtained from the resin of all pine trees is antiseptic, diuretic, rubefacient and vermifuge and is a valuable remedy used internally in the treatment of kidney and bladder complaints.

Chauhan (1999) reported that turpentine oils of *Pinus roxburghii* Sarg. is antiseptic and used as an expectorant in chronic bronchitis. They also reported the antibacterial activity of needle oils.

Dash and Gupta (1994) reported that wood oils of *Pinus roxburghii* Sarg. is used in Ayurvedic system of medicine as a nerve tonic, hemostatic, expectorant and diuretic. They also reported the use of this plant for skin diseases, ulcer, burns and cracks.

Ahmad et al., (1989) extracted tannins at different temperature with different solvent system from bark of *Pinus roxburghii* Sarg. They also measured the moisture content, viscosity, pH, and total dissolved solids of the extracts. The analysis by chemical techniques revealed that condensed tannins were present in the extracts.

Misra et al., (1988) reported monoterpenoids-4 on the optical purity of (+)-car-3-ene from *Pinus roxburghii* Sarg. and the source of racemization of (-)-methanol.

Bajracharya (1979) reported that wood oils of *Pinus roxburghii* Sarg. is used in Ayurvedic as a nerve tonic, haemostatic, expectorant and diuretic. They also reported the use of this plant for skin diseases, burns and cracks. Bark of *Pinus roxburghii* Sarg. is used for skin diseases and ulcers.

Chatterjee et al., (1977) reported the occurrence of hexacosylferulate in *Pinus roxburghii* Sarg. and its structure was derived from spectral measurements, chemical reactions and synthesis.
2.3 AIM AND OBJECTIVE

In the underdeveloped countries, traditional and herbal medicine practice scatters to most of the population because of accessibility, affordability as well as the time tested dependability. They still depend on herbal medicine because of the threat from side effects of the majority of the modern synthetic drugs. Plants have long been a very important source of new drugs and many plant species have been screened to see if they contain substances with therapeutic activity (Fabricant et al., 2001).

The plant Cedrus deodara Loud. and Pinus roxburghii Sarg. belonging to the family Pinaceae have long been known for their medicinal value. These plants have a long history of numerous traditional and ethnobotanical applications in diverse cultures (Shah, 2006; Husain et al., 2006; Kunwar et al., 2009).

The plant Cedrus deodara Loud. is used as diuretic, analgesic, diaphoretic and carminative. It is also used in inflammations, fever, bronchitis, itching, dyspepsia, insomnia, epilepsy, piles, skin disease, pulmonary disorder, urinary disorder, disorders of mind and ulcers (Agarwal & Rastogi, 1981; Kirtika & Basu, 1991; Nadkarni & Nadkarni, 1996). The researchers conducted in-vivo and in-vitro studies of Cedrus deodara Loud. and have reported its anti-inflammatory and analgesic (Shinde et al., 1999a), antioxidant (Saxena et al., 2010), anxiolytic, anticonvulsant (Viswanatha et al., 2009), antidiabetic (Shivanand et al., 2009), immunomodulatory (Shinde et al., 1999b) and anticancer (Singh et al., 2007) activities.

The plant Pinus roxburghii Sarg. is bitter, pungent, heating, oleaginous, intestinal antiseptic, purgative, carminative, expectorant, aphrodisiac, fattening, diuretic, anthelmintic, stimulant, analgesic and is used in diseases of the eye, liver, spleen, ear, throat, skin, bronchitis, diaphoresis, giddiness, ulcer, inflammation and itching (Kunwar et al., 2009; Chopra et al., 1986; Bajracharya, 1979; Dash & Gupta, 1994; Uniyal et al., 2006). The plant Pinus roxburghii Sarg. has been reported to have antidyshlipidemic and antioxidant (Puri et al., 2011), anti-inflammatory and analgesic (Kaushik et al., 2012a; Nemova et al., 2007) activities.

Review of the literature revealed that the memory enhancing, wound healing and antiulcer activities of these plants has not been subjected to scientific evaluation. Keeping these facts in mind we were trying to create a scientific base for the traditional use of these plants in neuropharmacology, wound healing, and ulcer and to identify the active fraction which is responsible for these activities.

The results emanating from the present study shall suggest the potential of Cedrus deodara Loud. and Pinus roxburghii Sarg. for the amelioration of amnesia, wound and ulcer. The overall goal of this study was to discover new herbal therapeutic agents with potential which could be explored as lead compounds for development as new drugs for the treatment of neurological disorder, wound and ulcer.